=> d his Inventor Search

(FILE 'HOME' ENTERED AT 07:47:32 ON 28 AUG 2000)

FILE 'CAPLUS, WPIDS, MEDLINE, BIOSIS' ENTERED AT 07:47:59 ON 28 AUG 2000
E VON EICHEL STREIBER C/AU
L1 191 S E3 OR E5-6 OR E8
E EICHEL STREIBER C/AU
L2 9 S E3-5

FILE 'CAPLUS, WPIDS, MEDLINE, BIOSIS' ENTERED AT 07:51:21 ON 28 AUG 2000
E BOQUET P/AU
L3 494 S E3-5
E THELESTAM M/AU
L4 276 S E3-5
L5 926 S L1 OR L2 OR L3 OR L4
SAVE L5 TEMP BURKE/A
L6 52155 S RAS
L7 47 S L5 AND L6
L8 224528 S TOXIN?
L9 35 S L7 AND L8
L10 0 S SORDELLI AND L7
L11 20 S SORDELLII AND L7
L12 35 S L9 OR L11
L13 20 DUP REM L12 (15 DUPLICATES REMOVED)

=> d bib ab 1-20

L13 ANSWER 1 OF 20 MEDLINE
AN 2000179883 MEDLINE
DN 20179883
TI Divergent roles for **Ras** and Rap in the activation of p38 mitogen-activated protein kinase by interleukin-1.
AU Palsson E M; Popoff M; **Thelestam M**; O'Neill L A
CS Department of Biochemistry, Biotechnology Institute, Trinity College, Dublin 2, Ireland.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Mar 17) 275 (11) 7818-25. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 200006
EW 20000603
AB We have found that lethal **toxin** from Clostridium **sordellii**, which specifically inactivates the low molecular weight G proteins **Ras**, Rap, and Rac, inhibits the activation of p38 mitogen-activated protein kinase (MAPK) by interleukin-1 (IL-1) in EL4.NOB-1 cells and primary fibroblasts. The target protein involved appeared to be **Ras**, because transient transfections with dominant negative RasN17 inhibited p38 MAPK activation by IL-1. Furthermore, transfections of cells with constitutively active RasVHa-activated p38 MAPK. Further evidence for **Ras** involvement came from the observation that IL-1 caused a rapid activation of **Ras** in the cells and from the inhibitory effects of the

Ras inhibitors manumycin A and damnacanthal. **Toxin** B from Clostridium difficile, which inactivates Rac, Cdc42, and Rho, was without effect. Dominant negative versions of Rac (RacN17) or Rap (Rap1AN17) did not inhibit the response. Intriguingly, transfection of cells with dominant negative Rap1AN17 activated p38 MAPK. Furthermore, constitutively active Rap1AV12 inhibited p38 MAPK activation by IL-1, consistent with Rap antagonizing **Ras** function. IL-1 also activated Rap in the cells, but with slower kinetics than **Ras**. Our studies therefore provide clear evidence using multiple approaches for **Ras** as a signaling component in the activation of p38 MAPK by IL-1, with Rap having an inhibitory effect.

L13 ANSWER 2 OF 20 MEDLINE
AN 2000383824 MEDLINE
DN 20292877
TI Activation of astroglial phospholipase D activity by phorbol ester involves ARF and Rho proteins.
AU Kotter K; Ji a S; **von Eichel-Streiber C**; Park J B; Ryu S H; Klein J
CS Department of Pharmacology, University of Mainz, Germany.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (2000 May 31) 1485 (2-3) 153-62. Journal code: A0W. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 200010
EW 20001002
AB Primary cultures of rat cortical astrocytes express phospholipase D (PLD) isoforms 1 and 2 as determined by RT-PCR and Western blot. Basal PLD activity was strongly (10-fold) increased by 4beta-phorbol-12beta,13alpha-dibutyrate (PDB) (EC(50): 56 nM), an effect which was inhibited by Ro 31-8220 (0.1-1 microM), an inhibitor of protein kinase C (PKC), and by brefeldin A (10-100 microg/ml), an inhibitor of ADP-ribosylating factor (ARF) activation. Pretreatment of the cultures with Clostridium difficile **toxin** B-10463 (0.1-1 ng/ml), which inactivates small G proteins of the Rho family, led to a breakdown of the astroglial cytoskeleton; concomitantly, PLD activation by PDB was reduced by up to 50%. In contrast, inactivation of proteins of the **Ras** family by Clostridium **sordellii** lethal **toxin** 1522 did not affect PLD activation. In parallel experiments, serum-induced PLD activation was sensitive to brefeldin A, but not to Ro 31-8220 and not to clostridial **toxins**. We conclude that, in astrocytes, the PLD isoform which is activated by phorbol ester requires PKC, ARF and Rho proteins for full activity and probably represents PLD1.

L13 ANSWER 3 OF 20 MEDLINE DUPLICATE 1
AN 1999253957 MEDLINE
DN 99253957
TI G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from Clostridium **sordellii** in HL-60 cells.
AU El Hadj N B; Popoff M R; Marvaud J C; Payrastre B; **Boquet P**; Geny B
CS INSERM U332, ICGM, 22 rue Mechain, 75014 Paris, France.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 May 14) 274 (20) 14021-31.

Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199908

AB Lethal **toxin** (LT) from Clostridium **sordellii** has been shown in HeLa cells to glucosylate and inactivate **Ras** and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that LT treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with LT. A similar dose response to the **toxin** was found when PLD activity was stimulated by phorbol 12-myristate 13-acetate via the protein kinase C pathway. The **toxin** effect on actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota **toxin** from Clostridium perfringens E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease observed in phosphatidylinositol 4,5-bisphosphate (PtdIns(4, 5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in LT-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. LT was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, Rac, and RalA, inactivated by LT and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as LT **toxin** (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, LT treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosol prepared from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase Calpha was decreased after LT treatment.
We conclude that in HL-60 cells, lethal **toxin** from C. **sordellii**, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications observed in HL-60 cells.

L13 ANSWER 4 OF 20 MEDLINE DUPLICATE 2

AN 1999214180 MEDLINE
DN 99214180
TI A novel cytotoxin from Clostridium difficile serogroup F is a functional hybrid between two other large clostridial cytotoxins.
AU Chaves-Olarte E; Low P; Freer E; Norlin T; Weidmann M; **von Eichel-Streiber C; Thelestam M**
CS Microbiology and Tumorbiology Center, Karolinska Institutet, S-171 77 Stockholm, Sweden.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Apr 16) 274 (16) 11046-52. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199907
EW 19990704
AB The large clostridial cytotoxins (LCTs) constitute a group of high molecular weight clostridial cytotoxins that inactivate cellular small GTP-binding proteins. We demonstrate that a novel LCT (TcdB-1470) from Clostridium difficile strain 1470 is a functional hybrid between "reference" TcdB-10463 and Clostridium **sordellii** TcsL-1522. It bound to the same specific receptor as TcdB-10463 but glucosylated the same GTP-binding proteins as TcsL-1522. All three **toxins** had equal enzymatic potencies but were equally cytotoxic only when microinjected. When applied extracellularly TcdB-1470 and TcdB-10463 were considerably more potent cytotoxins than TcsL-1522. The small GTP-binding protein R-**Ras** was identified as a target for TcdB-1470 and also for TcsL-1522 but not for TcdB-10463. R-**Ras** is known to control integrin-extracellular matrix interactions from inside the cell. Its glucosylation may be a major determinant for the cell rounding and detachment induced by the two R-**Ras**-attacking **toxins**. In contrast, fibroblasts treated with TcdB-10463 were arborized and remained attached, with phosphotyrosine containing structures located at the cell-to-cell contacts and beta3-integrin remaining at the tips of cellular protrusions. These components were absent from cells treated with the R-**Ras**-inactivating **toxins**. The novel hybrid **toxin** will broaden the utility of the LCTs for clarifying the functions of several small GTPases, now including also R-**Ras**.

L13 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3
AN 1999:350413 CAPLUS
DN 131:142850
TI Effects of cytotoxic necrotizing factor 1 and lethal **toxin** on actin cytoskeleton and VE-cadherin localization in human endothelial cell monolayers
AU Vouret-Craviari, Valerie; Grall, Dominique; Flatau, Gilles; Pouyssegur, Jacques; **Boquet, Patrice**; Van Obberghen-Schilling, Ellen
CS Centre de Biochimie, CNRS UMR 6543, Nice, 06108, Fr.
SO Infect. Immun. (1999), 67(6), 3002-3008 CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB Integrity of the vascular endothelium is largely dependent on endothelial cell shape and establishment of intercellular junctions. Certain pathogenic bacterial toxins alter the cytoskeletal architecture of

intoxicated cells by modulating the GTPase activity of p21 Rho family proteins. In the present study, the authors have analyzed the effect of Rho-directed toxins on the actin cytoskeleton and monolayer integrity of endothelial cells. Escherichia coli cytotoxic necrotizing factor 1 (CNF1) activated Rho in human umbilical vein endothelial cells (HUVEC). In confluent monolayers, CNF1 treatment induced prominent stress fiber formation without modifying peripheral localization of VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocked thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme (Clostridium botulinum) eliminated actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. Lethal toxin (Clostridium sordellii), an inhibitor of Rac as well as Ras and Rap, potently disrupted the actin microfilament system and monolayer integrity in HUVEC cultures.

RE.CNT 41
RE
(1) Baldacini, O; Toxicon 1992, V30, P129 CAPLUS
(2) Barbieri, B; Proc Soc Exp Biol Med 1981, V168, P204 CAPLUS
(3) Bette, P; Toxicon 1991, V29, P877 CAPLUS
(4) Braga, V; J Cell Biol 1997, V137, P1421 CAPLUS
(5) Braga, V; Mol Biol Cell 1999, V10, P9 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 6 OF 20 MEDLINE DUPLICATE 4
AN 1999346692 MEDLINE
DN 99346692
TI The actin-based motility of intracellular Listeria monocytogenes is not controlled by small GTP-binding proteins of the Rho- and **Ras**-subfamilies.
AU Ebel F; Rohde M; **von Eichel-Streiber C**; Wehland J; Chakraborty T
CS Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, Germany.. frank.ebel@mikrobio.med.uni-giessen.de
SO FEMS MICROBIOLOGY LETTERS, (1999 Jul 1) 176 (1) 117-24.
Journal code: FML. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199910
AB In this study, we analyzed whether the actin-based motility of intracellular Listeria monocytogenes is controlled by the small GTP-binding proteins of the Rho- and **Ras**-subfamilies. These signalling proteins are key regulatory elements in the control of actin dynamics and their activity is essential for the maintenance of most cellular microfilament structures. We used the Clostridium difficile **toxins** TcdB-10463 and TcdB-1470 to specifically inactivate these GTP-binding proteins. Treatment of eukaryotic cells with either of these **toxins** led to a dramatic breakdown of the normal actin cytoskeleton, but did not abrogate the invasion of epithelial cells by L.

monocytogenes and had no effect on the actin-based motility of this bacterial parasite. Our data indicate that intracellular Listeria reorganize the actin cytoskeleton in a way that circumvents the control mechanisms mediated by the members of the Rho- and **Ras** -subfamilies that can be inactivated by the TcdB-10463 and TcdB-1470 **toxins**.

L13 ANSWER 7 OF 20 MEDLINE
AN 2000131858 MEDLINE
DN 20131858
TI Bacterial **toxins** inhibiting or activating small GTP-binding proteins.
AU **Boquet P**
CS Institut National de la Sante et de la Recherche Medicale (INSERM), Faculte de Medecine, Nice, France.. boquet@unice.fr
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1999) 886 83-90. Ref: 49
Journal code: 5NM. ISSN: 0077-8923.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals; Cancer Journals
EM 200005
EW 20000501
AB Amino acids located on the switch 1 or switch 2 domains of small GTPases of the **Ras** and Rho family are targets of several bacterial **toxins**. Exoenzyme C3 from Clostridium botulinum ADP-ribosylates specifically Rho at R43 and prevents the recruitment of Rho on the cell membrane. This blocks the downstream effects of the Rho GTPase. However, exoenzyme C3 is not a **toxin**, and chimeric proteins fusing C3 with the B moiety of either diphtheria **toxin** or Pseudomonas aeruginosa exotoxin A have been produced to intoxicate cells with low concentration of C3. C. difficile **toxin** B modifies by glucosylation Rho on T37 and Rac and Cdc42 on T35. Glucosylation of Rho, Rac, and Cdc42 blocks the binding of these GTPases on their downstream effectors. C. **sordellii** lethal **toxin** modifies **Ras**, Rap, and Rac on T35 by glucosylation. Cytotoxic necrotizing factor 1 (CNF1), from uropathogenic Escherichia coli strains, deamidates Q63 of Rho into E63, thereby blocking the intrinsic or GAP-mediated GTPase of Rho. This allows permanent activation of Rho. Thus, Rho GTPases are targets for three different **toxin** activities. Molecular mechanisms of these **toxins** are discussed.

L13 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2000 ACS
AN 1999:658079 CAPLUS
DN 132:60177
TI The **Ras** superfamily of small GTP-binding proteins as targets for bacterial **toxins**
AU **Boquet, Patrice**
CS INSERM U452-Faculte de Medecine, Nice, 06107, Fr.
SO Compr. Sourceb. Bact. Protein Toxins (2nd Ed.) (1999), 27-44. Editor(s): Alouf, Joseph E.; Freer, John H. Publisher: Academic, London, UK.
CODEN: 68GNAV
DT Conference; General Review
LA English

AB A review with many refs. of the mol. activities of the Ras superfamily proteins and toxins interference with these mols. and of GTP-binding proteins as targets for toxins.
RE.CNT 159
RE
(1) Abo, A; Nature 1991, V353, P668 CAPLUS
(2) Adam, T; EMBO J 1996, V15, P3315 CAPLUS
(3) Adamson, P; J Biol Chem 1992, V267, P20033 CAPLUS
(5) Alb, J; Curr Opin Cell Biol 1996, V8, P534 CAPLUS
(6) Allen, W; J Cell Biol 1998, V141, P1147 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 9 OF 20 MEDLINE DUPLICATE 5
AN 1998184846 MEDLINE
DN 98184846
TI Specific inhibition of phorbol ester-stimulated phospholipase D by Clostridium **sordellii** lethal **toxin** and Clostridium difficile **toxin** B-1470 in HEK-293 cells. Restoration by Ral GTPases.
AU Schmidt M; Voss M; Thiel M; Bauer B; Grannass A; Tapp E; Cool R H; de Gunzburg J; **von Eichel-Streiber C**; Jakobs K H
CS Institut fur Pharmakologie, Universitatsklinikum Essen, D-45122 Essen, Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 27) 273 (13) 7413-22. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199806
AB Activation of m3 muscarinic acetylcholine receptor (mAChR), stably expressed in human embryonic kidney (HEK)-293 cells, leads to phospholipase D (PLD) stimulation, a process apparently involving Rho GTPases, as shown by studies with Clostridium botulinum C3 exoenzyme and Clostridium difficile **toxin** B (TcdB). Direct activation of protein kinase C (PKC) by phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), also induces PLD stimulation, which is additive to the mAChR action and which is only poorly sensitive to inactivation of Rho proteins by TcdB. To study whether **Ras**-like GTPases are involved in PLD regulation, we studied the effects of the TcdB variant TcdB-1470 and Clostridium **sordellii** lethal **toxin** (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by mAChR or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time- and concentration-dependent manner, without alteration in immunologically detectable PKC isozyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addition of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by

the addition of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addition of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TscL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddition of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

L13 ANSWER 10 OF 20 MEDLINE DUPLICATE 6
AN 1998249799 MEDLINE
DN 98249799
TI Rho protein inhibition blocks protein kinase C translocation and activation.
AU Hippenstiel S; Kratz T; Krull M; Seybold J; **von Eichel-Streiber C**; Suttorp N
CS Department of Internal Medicine, Justus-Liebig-University, Giessen, Germany.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Apr 28) 245 (3) 830-4.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199808
AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using Clostridium difficile **toxin** B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and Clostridium **sordellii** lethal **toxin** -1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as determined by PKC-activity measurements and Western blots for PKC alpha. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L13 ANSWER 11 OF 20 MEDLINE DUPLICATE 7
AN 1998336883 MEDLINE
DN 98336883
TI Small GTP-binding proteins of the Rho- and **Ras**-subfamilies are not involved in the actin rearrangements induced by attaching and effacing

Escherichia coli.
AU Ebel F; **von Eichel-Streiber C**; Rohde M; Chakraborty T
CS Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, Germany.. frank.ebel@mikrobio.med.uni-giessen.de
SO FEMS MICROBIOLOGY LETTERS, (1998 Jun 15) 163 (2) 107-12.
Journal code: FML. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199810
AB Attaching and effacing Escherichia coli (AEEC) are extracellular pathogens that induce the formation of actin-rich structures at their sites of attachment to eukaryotic host cells. We analysed whether small GTP-binding proteins of the Rho- and **Ras**-subfamilies, which control the cellular actin system, are essential for these bacterial-induced microfilament reorganizations. For this purpose we specifically inactivated them using the Clostridium difficile **toxins** TcdB-10463 and TcdB-1470. Such treatment led to a dramatic breakdown of the normal actin cytoskeleton, but did not abrogate the bacterial-induced actin rearrangements. Our data therefore indicate that the microfilament reorganizations induced by AEEC are independent of those small GTP-binding proteins that under normal conditions control the dynamics and maintenance of the actin cytoskeleton.

L13 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 8
AN 1997:533546 CAPLUS
DN 127:195467
TI Immunotoxin inactivation of **Ras** subfamily proteins and agents therefor
IN **Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica**
PA Boehringer Mannheim G.m.b.H., Germany; Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9727871	A1	19970807	WO 1997-EP426	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9715982	A1	19970822	AU 1997-15982	19970131
EP 877622	A1	19981118	EP 1997-902278	19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, FI
PRAI EP 1996-101469 19960202
WO 1997-EP426 19970131
AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the Ras proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of Clostridium sordellii toxin LT under conditions favoring inactivating of Ras by glucosylation of Ras' threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of toxin LT.

L13 ANSWER 13 OF 20 MEDLINE DUPLICATE 9
AN 97382287 MEDLINE
DN 97382287
TI Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a **toxin** that activates the Rho GTPase.
AU Fiorentini C; Fabbri A; Flatau G; Donelli G; Matarrese P; Lemichez E; Falzano L; **Boquet P**
CS Department of Ultrastructures, Istituto Superiore di Sanit`a, Viale Regina Elena 299, 00161, Rome, Italy.. MD2573@mclink.it
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Aug 1) 272 (31) 19532-7. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199710
AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein **toxin** from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the **toxin** into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of Rac, Cdc42, **Ras**, or Rab6) as demonstrated by a discrete increase in the apparent molecular weight of the molecule. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficile **toxin** B, which inactivates Rho but not those of Clostridium **sordellii** LT **toxin**, which inhibits **Ras** and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-associated phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PIP2) nor the phosphatidylinositol 3,4-bisphosphate (PI 3,4-P2) or 3,4,5-trisphosphate (PIP3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a **toxin** that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.

L13 ANSWER 14 OF 20 MEDLINE
AN 97459997 MEDLINE
DN 97459997

TI **Toxins** A and B from Clostridium difficile differ with respect to enzymatic potencies, cellular substrate specificities, and surface binding to cultured cells.
AU Chaves-Olarte E; Weidmann M; **Eichel-Streiber C; Thelestam M**
CS Microbiology and Tumorbiology Center (MTC), Karolinska institutet, S-171 77 Stockholm, Sweden.
SO JOURNAL OF CLINICAL INVESTIGATION, (1997 Oct 1) 100 (7) 1734-41. Journal code: HS7. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals; Cancer Journals
EM 199801
AB Clostridium difficile **toxins** A and B together are responsible for the symptoms of pseudomembranous colitis. Both **toxins** intoxicate cultured cells by the same mechanism but they differ in cytotoxic potency, **toxin** B being generally 1,000 times more potent than **toxin** A. Don and T84 cells were used to determine differences in the intoxication process exerted by both **toxins**. Three main differences were identified: (a) the specific binding of radiolabeled **toxins** to the cell surfaces correlated with the cytotoxic potency, (b) **toxin** B was found to have a 100-fold higher enzymatic activity than **toxin** A, and (c) **toxin** A was found to modify an additional substrate, Rap. The relative contribution of (a) and (b) to the difference in cytotoxic potency was determined by microinjection of the **toxins**. The differing enzymatic activities turned out to be the main determinant of the difference in cytotoxic potency, whereas the difference in binding contributes to a lesser degree. These findings are discussed in the context of the pathophysiological role of the **toxins**.

L13 ANSWER 15 OF 20 MEDLINE DUPLICATE 10
AN 97372557 MEDLINE
DN 97372557
TI Delineation of the catalytic domain of Clostridium difficile **toxin** B-10463 to an enzymatically active N-terminal 467 amino acid fragment.
AU Wagenknecht-Wiesner A; Weidmann M; Braun V; Leukel P; Moos M; **von Eichel-Streiber C**
CS Institut fur medizinische Mikrobiologie und Hygiene, Johannes Gutenberg-Universitat, Mainz, Germany.
SO FEMS MICROBIOLOGY LETTERS, (1997 Jul 1) 152 (1) 109-16. Journal code: FML. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-X92982
EM 199710
EW 19971003
AB In an attempt to directly approach the postulated toxic domain of Clostridium difficile's TcdB-10463, eight subclones of different size and locations in the N-terminal third of the **toxin** were generated. Expression of these **toxin** fragments was checked in Western blots and the enzymatic activity of the expressed proteins was analyzed by glucosylating **Ras** related small GTP-binding proteins. Two

polypeptides of 875 aa (TcdBc1-3) and 557 aa (TcdBc1-H) glucosylated their targets Rho, Rac and Cdc42 with the same activity and specificity as the holotoxin. In comparison 516 aa (TcdBc1-N) and 467 aa (TcdBc1-A) protein fragments exhibited highly reduced activity, while Tcdc1 and TcdB2-3 (aa 1-243 and 244-890, respectively) were enzymatically inactive. Our results indicate that all structures involved in the catalysis are located at several different sites within the 557 aa fully active fragment. The shortest enzymatically still active protein covers aa 1-467 and obviously fulfils all minimal requirements for glucosylation. The data support the postulated three domain model of 'large clostridial cytotoxins'.

L13 ANSWER 16 OF 20 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 11
AN 1996:256012 CAPLUS
DN 124:309937
TI **Ras**, Rap, and Rac small GTP-binding proteins are targets for Clostridium **sordellii** lethal **toxin** glucosylation
AU Popoff, Michel R.; Chaves-Olarte, Esteban; Lemichez, Emmanuel; **von Eichel-Streiber, Christoph**; **Thelestam, Monica**; Chardin, Pierre; Cussac, Didier; Antonny, Bruno; Chavrier, Philippe; et al.
CS Inst. Pasteur, Unite Toxines Microbiennes, Paris, 75724, Fr.
SO J. Biol. Chem. (1996), 271(17), 10217-24
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Lethal toxin (LT) from Clostridium sordellii is one of the high mol. mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that LT is a glucosyltransferase that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. LT glucosylates Ras, Rap, and Rac. In Ras, threonine at position 35 was identified as the target amino acid glucosylated by LT. Other related members of the Ras GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by LT. Incubation of serum-starved Swiss 3T3 cells with LT prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the toxin blocks Ras function in vivo. We also demonstrate that LT acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphol. LT is thus a powerful tool to inhibit Ras function in vivo.

L13 ANSWER 17 OF 20 MEDLINE
AN 97055675 MEDLINE
DN 97055675
TI Large clostridial cytotoxins--a family of glycosyltransferases modifying small GTP-binding proteins.
AU **von Eichel-Streiber C**; **Boquet P**; Sauerborn M; **Thelestam M**
CS Institut fur Medizinische Mikrobiologie und Hygiene, Johannes Gutenberg-Universitdt Mainz, Germany.. veichel@goofy.zdv.uni.mainz.de
SO TRENDS IN MICROBIOLOGY, (1996 Oct) 4 (10) 375-82. Ref: 55
Journal code: B1N. ISSN: 0966-842X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199704
AB Some Clostridium species produce ABX-type protein cytotoxins of high molecular weight. These **toxins** constitute the group of large clostridial cytotoxins (LCTs), which have homologous protein sequences, exert glycosyltransferase activity and modify GTP-binding proteins of the **Ras**-superfamily. These characteristics render the LCTs valuable tools for developmental and cell biologists.

L13 ANSWER 18 OF 20 MEDLINE
AN 92011877 MEDLINE
DN 92011877
TI The small GTP-binding protein Rho1p is localized on the Golgi apparatus and post-Golgi vesicles in Saccharomyces cerevisiae.
AU McCaffrey M; Johnson J S; Goud B; Myers A M; Rossier J; Popoff M R; Madaule P; **Boquet P**
CS Laboratoire de Physiologie Nerveuse, CNRS, Gif-sur-Yvette, France.
NC GM-39254 (NIGMS)
SO JOURNAL OF CELL BIOLOGY, (1991 Oct) 115 (2) 309-19.
Journal code: HMV. ISSN: 0021-9525.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199201
AB In Saccharomyces cerevisiae the **ras**-related protein Rho1p is essentially the only target for ADP-ribosylation by exoenzyme C3 of Clostridium botulinum. Using C3 to detect Rho1p in subcellular fractions, Rho1p was found primarily in the 10,000 g pellet (P2) containing large organelles; small amounts also were detected in the 100,000 g pellet (P3), and cytosol. When P2 organelles were separated in sucrose density gradients Rho1p comigrated with the Kex-2 activity, a late Golgi marker. Rho1p distribution was shifted from P2 to P3 in several mutants that accumulate post-Golgi vesicles. Rho1p comigrated with post-Golgi transport vesicles during fractionation of P3 organelles from wild-type or sec6 cells. Vesicles containing Rho1p were of the same size but different density than those bearing Sec4p, a **ras**-related protein located both on post-Golgi vesicles and the plasma membrane. Immunofluorescence microscopy detected Rho1p as a punctate pattern, with signal concentrated towards the cell periphery and in the bud. Thus, in S. cerevisiae Rho1p resides primarily in the Golgi apparatus, and also in vesicles that are likely to be early post-Golgi vesicles.

L13 ANSWER 19 OF 20 MEDLINE DUPLICATE 12
AN 90165949 MEDLINE
DN 90165949
TI Multiple small molecular weight guanine nucleotide-binding proteins in human erythrocyte membranes.
AU Damonte G; Sdraffa A; Zocchi E; Guida L; Polvani C; Tonetti M; Benatti U; **Boquet P**; De Flora A
CS Department of Biochemistry, University of Genoa, Italy.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1990 Feb 14) 166 (3)

1398-405.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199005
AB Native membranes from human erythrocytes contain the following G proteins which are ADP-ribosylated by a number of bacterial **toxins**: Gi alpha and Go alpha (pertussis **toxin**), Gs alpha (cholera **toxin**), and three proteins of 27, 26 and 22 kDa (exoenzyme C3 from Clostridium botulinum). Three additional C3 substrates (18.5, 16.5 and 14.5 kDa) appeared in conditions of unrestrained proteolysis during hemolysis. SDS-PAGE separation of erythrocyte membrane proteins followed by electroblotting and incubation of nitrocellulose sheets with radiolabeled GTP revealed consistently four GTP-binding proteins with Mr values of 27, 26, 22 and 21 kDa. Although a 22 kDa protein was immunochemically identified as **ras** p21, the C3 substrate of 22 kDa is a different protein probably identifiable with a rho gene product. Accordingly, at least five distinct small molecular weight guanine nucleotide-binding proteins, whose functions are so far undetermined, are present in native human erythrocyte membranes.

L13 ANSWER 20 OF 20 MEDLINE DUPLICATE 13
AN 88094413 MEDLINE
DN 88094413
TI Functional modification of a 21-kilodalton G protein when ADP-ribosylated by exoenzyme C3 of Clostridium botulinum.
AU Rubin E J; Gill D M; **Boquet P**; Popoff M R
CS Department of Molecular Biology and Microbiology, School of Medicine, Tufts University, Boston, Massachusetts 02111.
NC AI 16928 (NIAID)
AI 22145 (NIAID)
SO MOLECULAR AND CELLULAR BIOLOGY, (1988 Jan) 8 (1) 418-26.
Journal code: NGY. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198804
AB Exoenzyme C3 from Clostridium botulinum types C and D specifically ADP-ribosylated a 21-kilodalton cellular protein, p21.bot. Guanyl nucleotides protected the substrate against denaturation, which implies that p21.bot is a G protein. When introduced into the interior of cells, purified exoenzyme C3 ADP-ribosylated intracellular p21.bot and changed its function. NIH 3T3, PC12, and other cells rapidly underwent temporary morphological alterations that were in certain respects similar to those seen after microinjection of cloned **ras** proteins. When injected into Xenopus oocytes, C3 induced migration of germinal vesicles and potentiated the cholera **toxin**-sensitive augmentation of germinal vesicle breakdown by progesterone, also as caused by **ras** proteins. Nevertheless, p21.bot was immunologically distinct from p21ras.

USPATFULL

=> d que

L2 32 SEA FILE=USPATFULL ABB=ON ((C OR CLOSTRID?) (2W) SORDELLII)
L3 20119 SEA FILE=USPATFULL ABB=ON TOXIN# OR IMMUNOTOXIN# OR GLUCOSYLTR
ANSFER? OR RAS OR TRANSLOCATION DOMAIN OR CATALYTIC (2A) (PEPTIDE# OR POLYPEPTIDE# OR DOMAIN#)
L4 16 SEA FILE=USPATFULL ABB=ON L2 AND L3

=> d bib ab 1-16

L4 ANSWER 1 OF 16 USPATFULL
AN 2000:83853 USPATFULL
TI Multicomponent clostridial vaccines using saponin adjuvants
IN Roberts, David S., 1020 Rockhurst Dr., Lincoln, NV, United States 68510
PI US 6083512 20000704
AI US 1995-536970 19950929 (8)
RLI Continuation of Ser. No. WO 1994-US3395, filed on 29 Mar 1994 which is a continuation of Ser. No. US 1993-38428, filed on 29 Mar 1993, now abandoned
DT Utility
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Portner, Ginny Allen
LREP Richardson, Peter C.; Ginsburg, Paul G.; Koller, Alan L.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 713
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel multicomponent clostridial vaccine formulations using readily dispersible, non-depot adjuvants, such as saponin, are disclosed. The vaccines can be administered to cattle intramusculary or subcutaneously without the severe persistent local reactions, such as granulomas, abscesses, and scarring, normally seen with other multicomponent clostridial vaccines.

L4 ANSWER 2 OF 16 USPATFULL
AN 1999:75522 USPATFULL
TI Vaccine for clostridium botulinum neurotoxin
IN Williams, James A., Madison, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5919665 19990706
AI US 1995-405496 19950316 (8)
RLI Continuation-in-part of Ser. No. US 1994-329154, filed on 25 Oct 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-161907, filed on 2 Dec 1993, now patented, Pat. No. US 5601823 which is a continuation-in-part of Ser. No. US 1992-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 1989-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193, issued on 23 Mar 1993
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.; Assistant Examiner: Rabin,

Evelyn
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 31 Drawing Figure(s); 29 Drawing Page(s)
LN.CNT 9164
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes recombinant proteins derived from Clostridium botulinum toxins. In particular, soluble recombinant Clostridium botulinum type A toxin proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The soluble, endotoxin-free recombinant proteins are used as immunogens for the production of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin.

L4 ANSWER 3 OF 16 USPATFULL
AN 1999:75321 USPATFULL
TI Clostridium difficile **toxins** as mucosal adjuvants
IN Thomas, Jr., William D., Winchester, MA, United States
Monath, Thomas P., Harvard, MA, United States
Zhang, Zhenxi, Cambridge, MA, United States
Torres-Lopez, Francisco Javier, San Clemente, Mexico
Lei, Wende, Cambridge, MA, United States
Lyerly, David M., Radford, VA, United States
Moncrief, James S., Christiansburg, VA, United States
PA OraVax, Inc., Cambridge, MA, United States (U.S. corporation)
PI US 5919463 19990706
AI US 1995-543708 19951016 (8)
RLI Continuation-in-part of Ser. No. US 1995-499384, filed on 7 Jul 1995, now abandoned
DT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Masood, Khalid
LREP Clark & Elbing LLP
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 992
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention features methods and compositions for inducing protective and/or therapeutic immune responses to an antigen in a mammal. In these methods, an antigen is administered to the mammal with a **toxin** of a Clostridium (e.g., C. difficile), or a fragment or derivative thereof having adjuvant activity.

L4 ANSWER 4 OF 16 USPATFULL
AN 1999:40182 USPATFULL
TI Universal test systems and methods of use thereof for identifying multiple families of microorganisms
IN Godsey, James H., Folsom, CA, United States
Nothaft, Daniel M., Vacaville, CA, United States
PA Dade MicroScan Inc., West Sacramento, CA, United States (U.S. corporation)
PI US 5888760 19990330
AI US 1997-843634 19970410 (8)

DT Utility
EXNAM Primary Examiner: Leary, Louise N.
LREP Buckley, Linda M.; Buchanan, Robert L.; Ruszala, Lois K.
CLMN Number of Claims: 28
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1558
AB The present invention relates to a universal test systems and methods of use thereof for identifying a microorganism among at least two groups of widely divergent microorganisms. The universal test system comprises a predetermined combination of non-redundant biochemical tests comprising a substrate for at least one enzyme wherein the substrate, if acted on by the enzyme results in formation of a detectable product. Detectable products from the combination of biochemical tests are then used to identify the microorganism.

L4 ANSWER 5 OF 16 USPATFULL
AN 1999:24446 USPATFULL
TI Primers for the amplification of genes coding for the enterotoxin and the lecithinase of Clostridium perfringens and their application to the detection and numeration of these bacteriae
IN Fach, Patrick, Creteil, France
Guillou, deceased, Jean-Pierre, late of Chennevieres, France by Raymond Guillou, legal representative
Popoff, Michel, Clamart, France
PA Institut Pasteur, France (non-U.S. corporation)
Centre National D'Etudes Verterinaires et Alimentairescneva, France (non-U.S. government)
PI US 5874220 19990223
WO 9517521 19950629
AI US 1996-666405 19961108 (8)
WO 1994-EP4292 19941222
19961108 PCT 371 date
19961108 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1993-172026, filed on 22 Dec 1993, now patented, Pat. No. US 5538851
DT Utility
EXNAM Primary Examiner: Horlick, Kenneth R.
LREP Bierman, Muserlian and Lucas
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 1184
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Primers selected from the group consisting of SEQ ID Nos. 1, 2, 3, 4, 5, 6, 7 and 8 and an isolated nucleic acid encoding the C. Perfringens type .beta.-**toxin** .beta..sub.2 consisting of nucleotide sequence of SEQ ID No. 27 and the plasmids of the gene thereof.

L4 ANSWER 6 OF 16 USPATFULL
AN 1998:118999 USPATFULL
TI Recombinant clostridial **toxin** protein

IN Williams, James A., Madison, WI, United States
Kink, John A., Madison, WI, United States
Clemens, Christopher M., Madison, WI, United States
Carroll, Sean B., Cottage Grove, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5814477 19980929
AI US 1995-457048 19950601 (8)
RLI Division of Ser. No. US 1993-161907, filed on 2 Dec 1993, now patented, Pat. No. US 5601823 which is a continuation-in-part of Ser. No. US 1992-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser.
No. US 1989-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193, issued on 23 Mar 1993
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 14 Drawing Page(s)
LN.CNT 3080
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes methods for the production and purification of recombinant clostridial **toxin** proteins.

L4 ANSWER 7 OF 16 USPATFULL
AN 1998:98746 USPATFULL
TI Oligonucleotides for detecting bacteria and detection process
IN Nakayama, Tomoko, Osaka, Japan
Tada, Jun, Muko, Japan
Fukushima, Shigeru, Otsu, Japan
Ohashi, Tetsuo, Kyoto, Japan
PA Shimadzu Corporation, Kyoto, Japan (non-U.S. corporation)
PI US 5795717 19980818
AI US 1994-328710 19941025 (8)
PRAI JP 1994-30277 19940228
JP 1994-48174 19940318
DT Utility
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Rees, Dianne
LREP Birch, Stewart, Kolasch & Birch, LLP
CLMN Number of Claims: 7
ECL Exemplary Claim: 1,2
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 3242
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A synthetic oligonucleotide which is complementary to a nucleotide sequence of a gene selected from the group consisting of the Shiga **toxin** gene of Shigella species, the ipaH gene of Shigella species and EIEC, the invE gene of Shigella species and EIEC, the araC gene of Salmonella species, the Verocytotoxin-1 gene of EHEC or VTEC, the Verocytotoxin-2 gene of EHEC or VTEC, the toxic shock syndrome **toxin**-1 gene of Staphylococcus aureus, the ctx gene of Vibrio cholerae, and the enterotoxin gene of Clostridium perfringens; a method for detecting a bacterial strain by amplifying a region of the above gene by PCR using the above oligonucleotides as primers and detecting the amplified region; and a kit for the detection of the bacterial strain.

L4 ANSWER 8 OF 16 USPATFULL
AN 1998:64730 USPATFULL
TI Clostridium difficile **toxin** disease therapy
IN Williams, James A., Madison, WI, United States
Kink, John A., Madison, WI, United States
Clemens, Christopher M., Madison, WI, United States
Carroll, Sean B., Cottage Grove, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5762934 19980609
AI US 1995-456847 19950601 (8)
RLI Division of Ser. No. US 1993-161907, filed on 2 Dec 1993, now patented, Pat. No. US 5601823 which is a continuation-in-part of Ser. No. US 1992-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser.
No. US 1989-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193 And Ser. No. US 1992-842709, filed on 26 Feb 1992, now abandoned
which is a continuation-in-part of Ser. No. US -429791
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 3124
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes methods and compositions for treating humans and other animals intoxicated with at least one clostridial **toxin** by administration of antitoxin. In particular, the antitoxin directed against these **toxins** is produced in avian species. This avian antitoxin is designed so as to be orally administerable in therapeutic amounts and may be in any form (i.e., as a
solid or in aqueous solution).

L4 ANSWER 9 OF 16 USPATFULL
AN 1998:36359 USPATFULL
TI Treatment of Clostridium difficile induced disease
IN Kink, John A., Madison, WI, United States
Thalley, Bruce S., Madison, WI, United States
Stafford, Douglas C., Madison, WI, United States
Firca, Joseph R., Vernon Hills, IL, United States
Padhye, Nisha V., Madison, WI, United States
PA Ochidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5736139 19980407
AI US 1995-480604 19950607 (8)
RLI Continuation-in-part of Ser. No. US 1995-422711, filed on 14 Apr 1995 which is a continuation-in-part of Ser. No. US 1995-405496, filed on 16 Mar 1995 which is a continuation-in-part of Ser. No. US 1994-329154, filed on 24 Oct 1994 which is a continuation-in-part of Ser. No. US 1993-161907, filed on 2 Dec 1993, now patented, Pat. No. US 5601823 which is a continuation-in-part of Ser. No. US 1992-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 1989-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193, issued on 23

Mar 1993
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 28
ECL Exemplary Claim: 1
DRWN 55 Drawing Figure(s); 53 Drawing Page(s)
LN.CNT 11770
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present provides neutralizing antitoxin directed against C. difficile toxins. These antitoxins are produced in arian species using soluble recombinant C. difficile toxin proteins. The avian antitoxins are designed so as to be orally administrable in therapeutic amounts and may be in any form (i.e., as a solid or in aqueous solution). Solid forms of the antitoxin may comprise an enteric coating. These antitoxins are useful in the treatment of humans and other animals intoxicated with at least one bacterial toxin. The invention further provides vaccines capable of protecting a vaccinated recipient from the morbidity and mortality associated with C. difficile infection. These vaccines are useful for administration to humans and other animals at risk of exposure to C. difficile toxins.

L4 ANSWER 10 OF 16 USPATFULL
AN 1998:17427 USPATFULL
TI Clostridial **toxin** disease therapy
IN Carroll, Sean B., Cottage Grove, WI, United States
van Boldrik, Margaret B., Cottage Grove, WI, United States
Clemens, Christopher M., Madison, WI, United States
PA Ophidian Pharmaceuticals Inc., Madison, WI, United States (U.S. corporation)
PI US 5719267 19980217
AI US 1995-457890 19950601 (8)
RLI Division of Ser. No. US 1992-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 1989-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll,LLP
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 1310
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Treating humans and animals intoxicated with a bacterial **toxin** by administration of antitoxin. Avian antitoxin in an aqueous solution in therapeutic amount that is orally administrable.

L4 ANSWER 11 OF 16 USPATFULL
AN 97:120604 USPATFULL
TI Capsular polysaccharide immunomodulator
IN Tzianabos, Arthur O., Reading, MA, United States
Onderdonk, Andrew B., Westwood, MA, United States
Kasper, Dennis L., Newton Center, MA, United States
PA Brigham & Women's Hospital, Inc., Boston, MA, United States (U.S.

corporation)
PI US 5700787 19971223
AI US 1995-502865 19950714 (8)
RLI Continuation-in-part of Ser. No. US 1994-301271, filed on 2 Sep 1994
DT Utility
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Lee, Howard C.
LREP Wolf, Greenfield & Sacks, P.C.
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1475
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods and products for protecting against abscess formation associated with surgery, trauma or diseases that predispose the host to abscess formation are provided. Methods for forming immunomodulators and pharmaceutical compositions relating thereto also are provided. The products useful in the invention are polysaccharides including a repeat unit having a positively charged free amino group and a negatively charged group. The preferred polysaccharide is B. fragilis capsular polysaccharide A.

L4 ANSWER 12 OF 16 USPATFULL
AN 97:80916 USPATFULL
TI Inoculation of animals with dried, pelleted biological materials
IN Hansen, Richard D., Ankeny, IA, United States
Drake, James F., Minneapolis, MN, United States
PA InnoVac Co., Lincoln, NE, United States (U.S. corporation)
PI US 5665363 19970909
AI US 1996-712213 19960903 (8)
RLI Continuation of Ser. No. US 1994-356477, filed on 15 Dec 1994, now abandoned which is a continuation-in-part of Ser. No. US 1994-198836, filed on 18 Feb 1994, now abandoned
DT Utility
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Portner, Ginny Allen
LREP Merchant, Gould, Smith, Edell, Welter & Schmidt, P.A.
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 585
AB A method for vaccinating an animal by implanting subcutaneously an immune stimulating biologically active material into an animal with a biologically active pellet is described. Particularly described is the method of vaccinating an animal by implanting the pellet in the ear of an animal to eliminate edible tissue damage without inducing a "drooped ear" or "down ear".

L4 ANSWER 13 OF 16 USPATFULL
AN 97:12173 USPATFULL
TI Avian antitoxins to clostridium difficle **toxin** A
IN Williams, James A., Madison, WI, United States
Kink, John A., Madison, WI, United States
Clemens, Christopher M., Madison, WI, United States
Carroll, Sean B., Cottage Grove, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)

PI US 5601823 19970211
AI US 1993-161907 19931202 (8)
RLI Continuation-in-part of Ser. No. US 1992-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 1989-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 14 Drawing Page(s)
LN.CNT 3128
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes methods and compositions for treating humans and other animals intoxicated with at least one Clostridial **toxin** by administration of antitoxin. In particular, the antitoxin directed against these **toxins** is produced in avian species. This avian antitoxin is designed so as to be orally administerable in therapeutic amounts and may be in any form (i.e., as a solid or in aqueous solution).

L4 ANSWER 14 OF 16 USPATFULL
AN 96:65447 USPATFULL
TI Primers for the amplification of genes coding for the enterotoxin and the lecithinase of Clostridium perfringens and their application to the determination of the presence and numeration of these bacteriae
IN Fach, Patrick, Creteil, France
Guillou, Jean-Pierre, Chennevieres, France
Popoff, Michel, Clamart, France
PA Institut Pasteur and Cneva, France (non-U.S. corporation)
PI US 5538851 19960723
AI US 1993-172026 19931222 (8)
DT Utility
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Horlick, Kenneth R.
LREP Bierman and Muserlian
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 676
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Specific primers useful for the detection of the presence of lecithinase or enterotoxin genes or the presence of Clostridium perfringens bacteria in a sample by a polymerase chain reaction, particularly, in food sample or fecal samples.

L4 ANSWER 15 OF 16 USPATFULL
AN 93:61012 USPATFULL
TI Monoclonal antibodies specific for **Toxin** B of Clostridium difficile
IN Coughlin, Richard T., Leicester, MA, United States
Marciani, Dante J., Hopkinton, MA, United States
PA Cambridge Bioscience Corporation, Worcester, MA, United States (U.S.

corporation)
PI US 5231003 19930727
AI US 1990-522881 19900511 (7)
DT Utility
EXNAM Primary Examiner: Ceperley, Mary E.; Assistant Examiner: Bidwell, Carol E.
LREP Sterne, Kessler, Goldstein & Fox
CLMN Number of Claims: 9
ECL Exemplary Claim: 5
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 661
AB Monoclonal antibodies specific for **Toxin** B of Clostridium difficile are provided. Further, methods for making and using the antibodies are given, particularly the use of the antibodies for the detection of C. difficile.

L4 ANSWER 16 OF 16 USPATFULL
AN 86:35588 USPATFULL
TI Method for preventing or treating pseudo-membranous colitis
IN Hublot, Bernard, Paris, France
Levy, Rene H., Seattle, WA, United States
PA Laboratoires Biocodex, Montrouge, France (non-U.S. corporation)
PI US 4595590 19860617
AI US 1984-571523 19840117 (6)
DT Utility
EXNAM Primary Examiner: Schain, Howard E.
LREP Young & Thompson
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 165
AB The invention relates to a method for preventing or treating pseudo-membranous colitis in a patient submitted to antibiotic treatment
by means of Saccharomyces yeasts.

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=> d his

     (FILE 'HOME' ENTERED AT 14:45:50 ON 24 AUG 2000)

     FILE 'HCAPLUS' ENTERED AT 14:46:00 ON 24 AUG 2000
L1            90 S CLOSTRIDIUM SORDELLII
L2            38 S L1 (L) TOXIN#
L3          1583 S IMMUNOTOXIN#
L4         52513 S TOXIN#
L5         11350 S RAS
L6             7 S L1 AND L5
L7            43 S L1 AND (L3 OR L4)
L8            11 S L7 AND RAS/AB
L9            11 S L8 OR L6

     FILE 'REGISTRY' ENTERED AT 14:48:25 ON 24 AUG 2000
                E GLUCOSYLTRANSFERASE/CN
L10            1 S E3

     FILE 'HCAPLUS' ENTERED AT 14:48:41 ON 24 AUG 2000
L11         2439 S L10 OR GLUCOSYLTRANSFERASE#
L12            5 S L1 AND L11
L13          657 S GLUCOSYLATION
L14            7 S L1 AND L13
L15           14 S L14 OR L12 OR L9

=> d .ca hitstr l15 1-14


L15  ANSWER 1 OF 14  HCAPLUS  COPYRIGHT 2000 ACS
ACCESSION NUMBER:         2000:5127  HCAPLUS
DOCUMENT NUMBER:          132:147842
TITLE:                    Impact of amino acids 22-27 of Rho-subfamily GTPases
                          on glucosylation by the large clostridial
                          cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864
AUTHOR(S):                Muller, Stefani; Von Eichel-Streiber, Christoph;
Moos,
                          Michael
CORPORATE SOURCE:         Verfugungsbebaude fur Forschung und Entwicklung,
                          Institut fur Medizinische Mikrobiologie und Hygiene,
                          Johannes Gutenberg-Universitat, Mainz, 55101, Germany
SOURCE:                   Eur. J. Biochem. (1999), 266(3), 1073-1080
                          CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER:                Blackwell Science Ltd.
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   Here we report data describing some principles of the interaction between
     small GTP-binding proteins and large Clostridial cytotoxins (LCTs). Our
     investigation was based on the differential glucosylation of Rac1 vs.
RhoA
     by LCTs TcsL-1522, TcdB-1470 and TcdB-8864. Chimeric RhoA/Rac1 proteins
     and GTPases mutated at defined regions or single amino acids were used as
     substrates. Starting with chimeric Rac/Rho proteins we demonstrated that
     proteins contg. the N-terminal 73 amino acids of Rac1 (but not those of
     RhoA) were efficiently glucosylated. Within this stretch, three regions
     differ significantly in Rac1 and RhoA. Regions contg. amino acids 41-45
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and 50-54 had no effect on toxin induced glucosylation, whereas amino acids 22-27 had a drastic impact on the potential of all three toxins to covalently modify the GTPases. Point mutations K25T of RhoA (numbering according to Rac1) and K27A of Cdc42 significantly increased glucosylation
by the cytotoxins; introduction of lysines at the equiv. positions of Rac1
hindered modification. Our expts. demonstrate the influence of this charged residue on GTPase-LCT interactions. Amino acids 22-27 are part of
the transition between the .alpha.1-helix to the switch I region of small GTP-binding proteins; both are known structures for specificity detn. of the interactions with physiol. partners. Comparing these structures with data from our investigation we suggest that TcsL-1522, TcdB-1470 and TcdB-8864 mimic aspects of the physiol. interactions of small GTP-binding proteins.

CC 4-5 (Toxicology)

ST amino acid Rho GTPase **glucosylation** Clostridium cytotoxin

IT Proteins, specific or class
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(GTP-binding; impact of amino acids 22-27 of Rho-subfamily GTPases on **glucosylation** by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)

IT Toxins
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(cytotoxins; impact of amino acids 22-27 of Rho-subfamily GTPases on **glucosylation** by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene CDC42; impact of amino acids 22-27 of Rho-subfamily GTPases on **glucosylation** by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene rac1; impact of amino acids 22-27 of Rho-subfamily GTPases on **glucosylation** by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)

IT Clostridium difficile
Clostridium sordellii
Glucosylation
(impact of amino acids 22-27 of Rho-subfamily GTPases on **glucosylation** by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)

IT Rho protein (G protein)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(p21rhoA; impact of amino acids 22-27 of Rho-subfamily GTPases on **glucosylation** by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)

IT Mutation
(point; impact of amino acids 22-27 of Rho-subfamily GTPases on **glucosylation** by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)

IT 9059-32-9, GTPase
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(impact of amino acids 22-27 of Rho-subfamily GTPases on

glucosylation by large clostridial cytotoxins TcsL-1522, TcdB-1470 and TcdB-8864)
REFERENCE COUNT: 40
REFERENCE(S): (1) Abdul-Manan, N; Nature 1999, V399, P379 HCAPLUS
(2) Boguski, M; Nature 1993, V366, P643 HCAPLUS
(3) Boriack-Sjodin, P; Nature 1998, V394, P337 HCAPLUS
(4) Bradford, M; Anal Biochem 1976, V72, P248 HCAPLUS
(5) Chaves-Olarte, E; J Biol Chem 1999, V274, P11046 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 2 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:583555 HCAPLUS
DOCUMENT NUMBER: 131:209119
TITLE: Toxicologically active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**
INVENTOR(S): Aktories, Klaus; Hofmann, Fred
PATENT ASSIGNEE(S): Albert-Ludwigs-Universitaet Freiburg, Germany
SOURCE: Ger. Offen., 14 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19802569	A1	19990909	DE 1998-19802569	19980123

AB Fragment 1-546 of C. sordellii lethal toxin and an immunotoxin comprising this protein fused to a cell-binding moiety, such as a tumor cell-binding antibody or antibody fragment, are disclosed. The immunotoxin may addnl. contain a translocation signal, e.g., the translocation domain of Pseudomonas exotoxin A or of the Clostridium C2 toxin. The 1-546 fragment of the C. sordellii lethal toxin was found to have higher glucosyltransferase activity with **Ras** as substrate than did the wild-type lethal toxin.
IC ICM C12N009-10
ICS A61K038-45
ICI C12N009-10, C12R001-145
CC 1-6 (Pharmacology)
Section cross-reference(s): 3, 4
ST antitumor Clostridium lethal **toxin** fusion antibody **immunotoxin**
IT **Toxins**
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(C2,transport signal of Clostridium, **immunotoxin** contg.; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)
IT Antibodies
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological

study); PREP (Preparation); USES (Uses)
(antitumor, fusions with cytotoxin; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

IT **Clostridium sordellii**
(cytotoxin of; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

IT **Toxins**
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(cytotoxins, L, fusions with antitumor antibodies; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

IT **Toxins**
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(exotoxin A, transport signal of Pseudomonas, **immunotoxin** contg.; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

IT Immunoglobulins
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fragments, antitumor, fusions with cytotoxin; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

IT Drug delivery systems
(**immunotoxins**; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

IT Antitumor agents
(toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

IT 242136-30-7P
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; toxicol. active fragments of lethal **toxin** from **Clostridium sordellii** and their application in **immunotoxins**)

REFERENCE COUNT: 1
REFERENCE(S): (1) Datenbank Swissprat; Gene 1995, V161, P57

L15 ANSWER 3 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:351907 HCAPLUS
DOCUMENT NUMBER: 131:98722
TITLE: G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells
AUTHOR(S): El Hadj, Noomen Ben; Popoff, Michel R.; Marvaud, Jean-Christophe; Payrastre, Bernard; Boquet, Patrice; Geny, Blandine

CORPORATE SOURCE: INSERM U332, ICGM, Paris, 75014, Fr.
SOURCE: J. Biol. Chem. (1999), 274(20), 14021-14031
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Lethal toxin (LT) from Clostridium sordellii has been shown in HeLa cells to glucosylate and inactivate **Ras** and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that LT treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with LT. A similar dose response to the toxin was found when PLD activity was stimulated by phorbol 12-myristate 3-acetate via the protein kinase C pathway. The toxin effect on actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota toxin from Clostridium perfringens E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease obsd. in phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in Lt-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. LT was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, Rac, and RalA, inactivated by LT and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as LT toxin (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, LT treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosol prepd. from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase C.alpha. was decreased after LT treatment. We conclude that in HL-60 cells, lethal toxin from C. sordellii, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications obsd. in HL-60 cells.

CC 4-5 (Toxicology)

ST G protein phospholipase lethal **toxin** Clostridium
IT **Clostridium sordellii**
(G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT G proteins (guanine nucleotide-binding proteins)
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT Actins
Phosphatidylinositol 4,5-bisphosphate
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT Animal cell line
(HL-60; G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT Cytoplasm
(cytosol; G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT **Toxins**
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(lethal; G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT Transport proteins
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(phosphatidylinositol transfer protein; G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT 9001-87-0, Phospholipase D 37205-54-2, Phosphatidylinositol 4-kinase 59977-48-9, Phosphoinositide kinase 104645-76-3, Phosphatidylinositol 4-phosphate 5-kinase 115926-52-8, Phosphatidylinositol 3-kinase
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
IT 141436-78-4
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(.alpha.; G-protein-stimulated phospholipase D activity is inhibited by lethal **toxin** from **Clostridium sordellii** in HL-60 cells)
REFERENCE COUNT: 57
REFERENCE(S):
(1) Aktories, K; Mol Cell Biochem 1994, V138, P167 HCAPLUS
(3) Auger, K; Cell 1989, V57, P167 HCAPLUS

(5) Bradford, M; Anal Biochem 1976, V72, P248 HCAPLUS
(6) Chong, L; Cell 1994, V79, P507 HCAPLUS
(7) Choudhury, S; Cancer Lett 1996, V109, P149 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 4 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:62268 HCAPLUS
DOCUMENT NUMBER: 130:206160
TITLE: Inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes
AUTHOR(S): Rime, Helene; Talbi, Nabila; Popoff, Michel R.; Suziedelis, Kestutis; Jessus, Catherine; Ozon, Rene
CORPORATE SOURCE: Laboratoire de Physiologie de la Reproduction, INRA/ESA-CNRS 7080, Universite Pierre et Marie Curie, Paris, 75252, Fr.
SOURCE: Dev. Biol. (1998), 204(2), 592-602
CODEN: DEBIAO; ISSN: 0012-1606
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The lethal toxin (LT) from Clostridium sordellii is a glucosyltransferase that modifies and inhibits small G proteins of the **Ras** family, **Ras** and Rap, as well as Rac proteins. LT induces cdc2 kinase activation and germinal vesicle breakdown (GVBD) when microinjected into full-grown Xenopus oocytes. Toxin B from Clostridium difficile, that glucosylates and inactivates Rac proteins, does not induce cdc2 activation, indicating that proteins of the **Ras** family, **Ras** and(or) Rap, neg. regulate cdc2 kinase activation in Xenopus oocyte. In oocyte exts., LT catalyzes the incorporation of [14C]glucose into a group of proteins of 23 kDa and into 1 protein of 27 kDa. The 23-kDa proteins are recognized by anti-Rap1 and anti-Rap2 antibodies whereas the 27-kDa protein is recognized by several anti-**Ras** antibodies and probably corresponds to K-**Ras**. Microinjection of LT into oocytes together with UDP-[14C]glucose results in a glucosylation pattern similar to the in vitro glucosylation, indicating that the 23- and 27-kDa proteins are in vivo substrates of LT. In vivo time-course anal. reveals that the 27-kDa protein glucosylation is completed within 2 h, well before cdc2 kinase activation, whereas the 23-kDa proteins are partially glucosylated at GVBD. This observation suggests that the 27-kDa **Ras** protein could be the in vivo target of LT allowing cdc2 kinase activation. Interestingly, inactivation of **Ras** proteins does not prevent the phosphorylation of c-Raf1 and the activation of MAP kinase that occurs normally around GVBD. (c) 1998 Academic Press.

CC 4-5 (Toxicology)
ST G protein inhibition Clostridium lethal **toxin**; kinase cdc2 MAP Xenopus oocyte activation
IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(23,000-mol.-wt.; inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
IT Proteins (specific proteins and subclasses)

RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(27,000-mol.-wt.; inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
IT Proteins (specific proteins and subclasses)
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(RAP (receptor-assocd. protein); inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
IT **Clostridium sordellii**
Germinal vesicle
Glucosylation
Oocyte
Xenopus laevis
(inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
IT **Ras** proteins
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
IT **Toxins**
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(lethal; inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
IT G proteins (guanine nucleotide-binding proteins)
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(small; inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
IT 142243-02-5, MAP kinase 143375-65-9, Cdc2 kinase
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(inhibition of small G proteins by **Clostridium sordellii** lethal **toxin** activates cdc2 and MAP kinase in Xenopus oocytes)
REFERENCE COUNT: 55
REFERENCE(S): (1) Allende, C; FEBS Lett 1988, V234, P426 HCAPLUS
(2) Andeol, Y; Dev Biol 1990, V139, P24 HCAPLUS
(3) Anderson, C; J Virol 1973, V12, P241 HCAPLUS
(4) Barrett, C; Mol Cell Biol 1990, V10, P310 HCAPLUS
(5) Baum, E; Oncogene 1990, V5, P763 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 5 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:223590 HCAPLUS
DOCUMENT NUMBER: 128:318211

TITLE: Specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii** lethal **toxin** and Clostridium difficile **toxin** B-1470 in HEK-293 cells. Restoration by Ral GTPases

AUTHOR(S): Schmidt, Martina; Voss, Matthias; Thiel, Markus; Bauer, Bettina; Grannass, Andreas; Tapp, Eva; Cool, Robbert H.; De Gunzburg, Jean; Von Eichel-Streiber, Christoph; Jakobs, Karl H.

CORPORATE SOURCE: Universitatsklinikum Essen, Institut fur Pharmakologie, Essen, D-45122, Germany

SOURCE: J. Biol. Chem. (1998), 273(13), 7413-7422
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB To study whether **Ras**-like GTPases are involved in phospholipase D (PLD) regulation, we studied the effects of the Clostridium difficile toxin B (TcdB) variant TcdB-1470 and Clostridium sordellii lethal toxin (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by m3 muscarinic acetylcholine receptor (mAChR) or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time-and concn.-dependent manner, without alteration in immunol. detectable protein kinase C (PKC) isoenzyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addn. of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addn. of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addn. of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TscL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddn. of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

CC 4-5 (Toxicology)

ST phospholipase D Clostridium **toxin** Ral GTPase

IT **Toxins**
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)

(B; specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii** lethal **toxin** and Clostridium difficile **toxin** B-1470 in HEK-293 cells in relation to Ral GTPases)

IT **Toxins**
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(lethal; specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii** lethal **toxin** and Clostridium difficile **toxin** B-1470 in HEK-293 cells in relation to Ral GTPases)

IT Animal cells
Clostridium difficile
Clostridium sordellii
(specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii** lethal **toxin** and Clostridium difficile **toxin** B-1470 in HEK-293 cells in relation to Ral GTPases)

IT G proteins (guanine nucleotide-binding proteins)
Muscarinic receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii** lethal **toxin** and Clostridium difficile **toxin** B-1470 in HEK-293 cells in relation to Ral GTPases)

IT 9001-87-0, Phospholipase D 9059-32-9, GTPase 141436-78-4, Protein kinase C
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii** lethal **toxin** and Clostridium difficile **toxin** B-1470 in HEK-293 cells in relation to Ral GTPases)

L15 ANSWER 6 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:130788 HCAPLUS
DOCUMENT NUMBER: 128:253966
TITLE: Chimeric clostridial cytotoxins: identification of the N-terminal region involved in protein substrate recognition
AUTHOR(S): Hofmann, Fred; Busch, Christian; Aktories, Klaus
CORPORATE SOURCE: Institute fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Freiburg, D-79104, Germany
SOURCE: Infect. Immun. (1998), 66(3), 1076-1081
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Clostridium sordellii lethal toxin is a member of the family of large clostridial cytotoxins that glucosylate small GTPases. In contrast to Clostridium difficile toxins A and B, which exclusively modify Rho subfamily proteins, C. sordellii lethal toxin also glucosylates **Ras** subfamily proteins. By deletion anal. and construction of chimeric fusion proteins of C. sordellii lethal toxin and C. difficile toxin B, we localized the enzyme activity of the lethal toxin to the N terminus of the holotoxin and identified the region involved in protein

substrate specificity. The toxin fragment of the N-terminal 546 amino acid residues of C. sordellii lethal toxin glucosylated Rho and **Ras** subfamily proteins, as the holotoxin did. Deletion of a further 30 amino acid residues from the C terminus of this active fragment drastically reduced glucotransferase activity and blocked glucohydrolase activity. Exchange of amino acid residues 364 through 516 of lethal toxin for those in the active toxin B fragment (1 to 546) allowed glucosylation of **Ras** subfamily proteins. In contrast, the chimera with amino acids 1 to 364 from toxin B, 365 to 468 from lethal toxin, and 469 to 546 from toxin B exhibited markedly reduced modification of **Ras** subfamily proteins, whereas modification of Rac and Cdc42 was hardly changed. The data indicate that the region of amino acid residues 364 through 516 primarily defines the substrate specificity of C. sordellii lethal toxin.

CC 4-5 (Toxicology)

ST Clostridium cytotoxin protein substrate recognition; lethal **toxin** Clostridium **Ras** protein

IT **Clostridium sordellii**
(chimeric clostridial cytotoxins and identification of N-terminal region involved in protein substrate recognition)

IT **Ras** proteins
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(chimeric clostridial cytotoxins and identification of N-terminal region involved in protein substrate recognition)

IT **9031-48-5, Glucosyltransferase** 9033-06-1, Glucohydrolase
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(chimeric clostridial cytotoxins and identification of N-terminal region involved in protein substrate recognition)

IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(chimeric clostridial cytotoxins and identification of N-terminal region involved in protein substrate recognition)

RN 9031-48-5 HCAPLUS

CN Glucosyltransferase (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

L15 ANSWER 7 OF 14 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1997:533546 HCAPLUS

DOCUMENT NUMBER: 127:195467

TITLE: **Immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor

INVENTOR(S): Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica

PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany; Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica

SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9727871	A1	19970807	WO 1997-EP426	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9715982	A1	19970822	AU 1997-15982	19970131
EP 877622	A1	19981118	EP 1997-902278	19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRIORITY APPLN. INFO.:			EP 1996-101469	19960202
			WO 1997-EP426	19970131

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras** proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of Clostridium sordellii toxin LT under conditions favoring inactivating of **Ras** by glucosylation of **Ras'** threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of toxin LT.

IC ICM A61K038-45
ICS A61K047-48; A61K048-00; C07K016-46; C12N009-10; C07K019-00

CC 63-5 (Pharmaceuticals)
Section cross-reference(s): 1, 15

ST **immunotoxin** inactivation **Ras** protein antitumor

IT **Clostridium sordellii**
(LT **toxin** of; **immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)

IT c-**ras** protein
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**glucosylation** of; **immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)

IT Antitumor agents
Colon tumor inhibitors
Genetic vectors
Retroviral vectors
Virus vectors
(**immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)

IT **Immunotoxins**
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)

IT **ras** gene (animal)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)

IT Pancreatic tumors
(inhibitors; **immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)
IT Heat labile enterotoxin
RL: BAC (Biological activity or effector, except adverse); PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(of **Clostridium sordellii**; **immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)
IT **Glucosylation**
(of **Ras**; **immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)
IT Antitumor agents
(pancreatic; **immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)
IT Antibodies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(target cell-specific; **immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)
IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(**immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)
IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(**immunotoxin** inactivation of **Ras** subfamily proteins and agents therefor)
RN 9031-48-5 HCAPLUS
CN Glucosyltransferase (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

L15 ANSWER 8 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:518986 HCAPLUS
DOCUMENT NUMBER: 127:132163
TITLE: Enterotoxin A and cytotoxin B (Clostridium difficile)
AUTHOR(S): von Eichel-Streiber, Christoph
CORPORATE SOURCE: Verfugungsgebaude Forschung Entwicklung, Institut Medinzinische Mikrobiologie Hygiene, Mainz, 55111, Germany
SOURCE: Guideb. Protein Toxins Their Use Cell Biol. (1997), 72-77. Editor(s): Rappuoli, Rino; Montecucco, Cesare.
Oxford University Press: Oxford, UK.
CODEN: 64UWAW
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Clostridium difficile enterotoxin A (TcdA, 308 kDa) and cytotoxin B (TcdB, 270 kDa) belong to the group of large clostridial cytotoxins (LCT). The toxins are secreted into the culture supernatant of the growing bacteria,

specifically bind to eukaryotic cells, and are then taken up by receptor mediated endocytosis. Intracellularly they monoglucosylate small GTP-binding proteins, mainly of the Rho subfamily, at their effector domain. The GTPases are thus functionally inactivated, the result is a breakdown of the cellular actin stress fibers, a block of cytokinesis, but not a loss of vitality of the cells.

CC 4-5 (Toxicology)

IT Clostridium difficile
Clostridium sordellii
Cytokinesis
Cytotoxicity
Endocytosis
Growth (microbial)
(enterotoxin A and cytotoxin B (Clostridium difficile) in relation to cytotoxicity, purifn., antibody formation, and uses)

IT **Glucosylation**
(mono; enterotoxin A and cytotoxin B (Clostridium difficile) in relation to cytotoxicity, purifn., antibody formation, and uses)

L15 ANSWER 9 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:510330 HCAPLUS
DOCUMENT NUMBER: 127:172444
TITLE: Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a **toxin** that activates the Rho GTPase
AUTHOR(S): Fiorentini, Carla; Fabbri, Alessia; Flatau, Gilles; Donelli, Gianfranco; Matarrese, Paola; Lemichez, Emmanuel; Falzano, Loredana; Boquet, Patrice
CORPORATE SOURCE: Dep. Ultrastructures, Inst. Superiore Sanita, Rome, 00161, Italy
SOURCE: J. Biol. Chem. (1997), 272(31), 19532-19537
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein toxin from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the toxin into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of Rac, Cdc42, **Ras**, or Rab6) as demonstrated by a discrete increase in the apparent mol. wt. of the mol. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficult toxin B, which inactivates Rho but not those of Clostridium sordellii LT toxin, which inhibits **Ras** and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-assocd. phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PI 3,4-P2) or 3,4,5-trisphosphate (PIP3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation

of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a toxin that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.
CC 4-5 (Toxicology)
IT **Toxins**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(B, Clostridium difficile; Escherichia coli cytotoxic necrotizing factor 1 activation of Rho GTPase)
IT **Toxins**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(**Clostridium sordellii** lethal **toxin**; Escherichia coli cytotoxic necrotizing factor 1 activation of Rho GTPase)
IT **Toxins**
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(Escherichia coli cytotoxic necrotizing factor 1; Escherichia coli cytotoxic necrotizing factor 1 activation of Rho GTPase)
IT **Clostridium sordellii**
(lethal **toxin**; Escherichia coli cytotoxic necrotizing factor 1 activation of Rho GTPase)
IT Clostridium difficile
(**toxin** B; Escherichia coli cytotoxic necrotizing factor 1 activation of Rho GTPase)

L15 ANSWER 10 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:761992 HCAPLUS
DOCUMENT NUMBER: 126:43823
TITLE: Difference in protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**
AUTHOR(S): Genth, Harald; Hofmann, Fred; Selzer, Joerg; Aktories, Klaus; Just, Ingo
CORPORATE SOURCE: Institut fuer Pharmakologie der Albert-Ludwigs-Universitaet Freiburg, Freiburg, D-79104, Germany
SOURCE: Biochem. Biophys. Res. Commun. (1996), 229(2), 370-374
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Here we report that hemorrhagic toxin (HT), which is coexpressed with lethal toxin, is also a glucosyltransferase. Whereas lethal toxin glycosylates the Rho subfamily proteins Rac and Cdc42 and the **Ras** subfamily proteins H-**Ras** and Rap, the substrate specificity of HT is strictly confined to the Rho subfamily proteins Rho, Rac and Cdc42. Comparable to lethal toxin, transferase activity of HT is stimulated by Mn2+. Acceptor amino acid in Rho was identified by mutagenesis as threonine-37. C. sordellii HT is a novel member of the family of clostridial mono-glucosyl-transferases, a family which modifies the Rho and **Ras** of GTPases.
CC 4-5 (Toxicology)
ST protein substrate hemorrhagic lethal **toxin** Clostridium
IT Proteins (specific proteins and subclasses)

RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene Arf1; protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene CDC42; protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene Ran; protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene rab5; protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene rac1; protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT **Toxins**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(lethal; protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT Rho protein (G protein)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(p21rhoA; protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT **Clostridium sordellii**
(protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT Hemorrhagins
Proteins (general), biological studies
p21c-Ha-**ras** protein
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT Divalent cations
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii** in relation to **glucosyltransferase** activity and divalent cations)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(smg-21 (small-mol.-wt., 21,000-mol.-wt.); protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii**)

IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)

(protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii** in relation to **glucosyltransferase** activity)

IT 7439-95-4, Magnesium, biological studies 7439-96-5, Manganese, biological studies 7440-48-4, Cobalt, biological studies 7440-50-8, Copper, biological studies 7440-66-6, Zinc, biological studies 7440-70-2, Calcium, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii** in relation to **glucosyltransferase** activity and divalent cations)

IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(protein substrate specificity between hemorrhagic **toxin** and lethal **toxin** from **Clostridium sordellii** in relation to **glucosyltransferase** activity)

RN 9031-48-5 HCAPLUS
CN Glucosyltransferase (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

L15 ANSWER 11 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:606610 HCAPLUS
DOCUMENT NUMBER: 125:240634
TITLE: The **Ras**-related protein Ral is monoglucosylated by **Clostridium sordellii** lethal **toxin**
AUTHOR(S): Hofmann, Fred; Rex, Gundula; Aktories, Klaus; Just, Ingo
CORPORATE SOURCE: Institut fuer Pharmakologie und Toxikologie, AlbertLudwigs-Universitaet Freiburg, Freiburg, D-79104, Germany
SOURCE: Biochem. Biophys. Res. Commun. (1996), 227(1), 77-81
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We report here on lethal toxin (LT) produced by C. sordellii strain 6018 which glucosylates in addn. to Rac, **Ras** and Rap the Ral protein. LT from strain VPI9048 however does not glucosylate Ral. Besides recombinant Ral, cellular Ral is also substrate. In the GDP-bound form, Ral is a superior substrate to the GTP form. Acceptor amino acid for glucose is threonine-46 which is equiv. to threonine-35 in H-**Ras** located in the effector region. The Ral-glucosylating toxin is a novel isoform of **Ras**-modifying clostridial cytotoxins.

CC 4-5 (Toxicology)
ST protein Ral **glucosylation** Clostridium lethal **toxin**
IT Proteins, specific or class
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Ral; protein Ral monoglucosylation by **Clostridium sordellii** lethal **toxin**)
IT **Toxins**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(lethal; protein Ral monoglucosylation by **Clostridium sordellii** lethal **toxin**)
IT **Clostridium sordellii**

(protein Ral monoglucosylation by **Clostridium sordellii** lethal **toxin**)
IT Glycosidation
(glucosidation, protein Ral monoglucosylation by **Clostridium sordellii** lethal **toxin**)

L15 ANSWER 12 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:256012 HCAPLUS
DOCUMENT NUMBER: 124:309937
TITLE: **Ras**, Rap, and Rac small GTP-binding proteins are targets for **Clostridium sordellii** lethal **toxin glucosylation**
AUTHOR(S): Popoff, Michel R.; Chaves-Olarte, Esteban; Lemichez, Emmanuel; von Eichel-Streiber, Christoph; Thelestam, Monica; Chardin, Pierre; Cussac, Didier; Antonny, Bruno; Chavrier, Philippe; et al.
CORPORATE SOURCE: Inst. Pasteur, Unite Toxines Microbiennes, Paris, 75724, Fr.
SOURCE: J. Biol. Chem. (1996), 271(17), 10217-24
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lethal toxin (LT) from Clostridium sordellii is one of the high mol. mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that LT is a glucosyltransferase that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. LT glucosylates **Ras**, Rap, and Rac. In **Ras**, threonine at position 35 was identified as the target amino acid glucosylated by LT. Other related members of the **Ras** GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by LT. Incubation of serum-starved Swiss 3T3 cells with LT prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the toxin blocks **Ras** function in vivo. We also demonstrate that LT acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphol. LT is thus a powerful tool to inhibit **Ras** function in vivo.
CC 4-5 (Toxicology)
ST GTP binding protein Clostridium lethal **toxin**; **glucosylation** GTP protein Clostridium lethal **toxin**
IT **Clostridium sordellii**
(**Clostridium sordellii** lethal **toxin glucosylation** targets)
IT Proteins, specific or class
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene Arf1; **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT Proteins, specific or class
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(GTP-binding, **Clostridium sordellii** lethal **toxin glucosylation** targets)

IT **Toxins**
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BIOL (Biological study); PROC (Process)
(entero-, LT, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene CDC42, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene rab6, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene rac1, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT Proteins, specific or class
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene ral, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene rap2, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT Glycosidation
(glucosidation, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(p21Ha-**ras**, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(p21rhoA, **Clostridium sordellii** lethal **toxin glucosylation** targets)
IT 72-19-5, Threonine, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(of **Ras** protein in position 35; **Clostridium sordellii** lethal **toxin glucosylation** targets)

L15 ANSWER 13 OF 14 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:256001 HCAPLUS
DOCUMENT NUMBER: 124:309936
TITLE: Inactivation of **Ras** by **Clostridium sordellii** lethal **toxin**-catalyzed **glucosylation**
AUTHOR(S): Just, Ingo; Selzer, Joerg; Hofmann, Fred; Green, Gaynor A.; Aktories, Klaus
CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Univ. Freiburg, Freiburg, D-79104, Germany
SOURCE: J. Biol. Chem. (1996), 271(17), 10149-53
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The lethal toxin (LT) from Clostridium sordellii belongs to the family of

large clostridial cytotoxins causing morphol. alterations in cultured cell lines accompanied by destruction of the actin cytoskeleton. C.. sordellii LT exhibits 90% homol. to Clostridium difficile toxin B, which has been recently identified as a monoglucosyltransferase (1995). We report here that LT too is a glucosyltransferase, which uses UDP-glucose as cosubstrate to modify low mol. mass GTPases. LT selectively modified Rac and **Ras**, whereas the substrate specificity of toxin B is confined to the Rho subfamily proteins Rho, Rac, and Cdc42, which participate in the regulation of the actin cytoskeleton. In Rac, both toxin B and LT share the same acceptor amino acid, threonine 35. Glucosylation of **Ras** by LT results in inhibition of the epidermal growth factor-stimulated p42/p44 MAP-kinase signal pathway. LT is the first bacterial toxin to inactivate **Ras** in intact cells.

CC 4-5 (Toxicology)

ST **Ras** gene Clostridium lethal **toxin** **glucosylation**

IT **Clostridium sordellii**
(**Ras** inactivation by **Clostridium sordellii** lethal **toxin**-catalyzed **glucosylation**)

IT **Toxins**
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(entero-, LT, inactivation of **Ras** by **Clostridium sordellii** lethal **toxin**-catalyzed **glucosylation**)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene c-**ras**, **Ras** inactivation by **Clostridium sordellii** lethal **toxin** -catalyzed **glucosylation**)

IT Glycosidation
(glucosidation, inactivation of **Ras** by **Clostridium sordellii** lethal **toxin**-catalyzed **glucosylation**)

IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(inactivation of **Ras** by **Clostridium sordellii** lethal **toxin**-catalyzed **glucosylation**)

IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(inactivation of **Ras** by **Clostridium sordellii** lethal **toxin**-catalyzed **glucosylation**)

RN 9031-48-5 HCAPLUS

CN Glucosyltransferase (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

L15 ANSWER 14 OF 14 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1996:188145 HCAPLUS

DOCUMENT NUMBER: 124:223328

TITLE: UDP-glucose deficiency in a mutant cell line protects against **glucosyltransferase** toxins from

Clostridium difficile and **Clostridium sordellii**

AUTHOR(S): Chaves-Olarte, Esteban; Florin, Inger; Boquet, Patrice; Popoff, Michel; von Eichel-Streiber, Christoph; Thelestam, Monica

CORPORATE SOURCE: Microbiology & Tumorbiology Center (MTC), Karolinska Inst., Stockholm, S-171 77, Swed.

SOURCE: J. Biol. Chem. (1996), 271(12), 6925-32
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors have previously isolated a fibroblast mutant cell with high resistance to the two Rho-modifying glucosyl-transferase toxins A and B of Clostridium difficile. The authors demonstrate here a low level of UDP-glucose in the mutant, which explains its toxin resistance since: (i) to obtain a detectable toxin B-mediated Rho modification in lysates of mutant cells, addn. of UDP-glucose was required, and it promoted the Rho modification dose-dependently; (ii) high pressure liq. chromatog. anal. of nucleotide exts. of cells indicated that the level of UDP-glucose in the mutant (0.8 nmol/106 cells) was lower than in the wild type (3.7 nmol/106 cells); and (iii) sensitivity to toxin B was restored upon microinjection of UDP-glucose. Using the mutant as indicator cell the authors also found that the related Clostridium sordellii lethal toxin is a glucosyltransferase which requires UDP-glucose as a cofactor. Like toxin B it glucosylated 21-23-kDa proteins in cell lysates, but Rho was not a substrate for lethal toxin.

CC 4-5 (Toxicology)

ST UDP glucose **glucosyltransferase** Clostridium toxin

IT Clostridium difficile
Clostridium sordellii
(UDP-glucose deficiency in mutant cell line and protection against **glucosyltransferase** toxins from Clostridium difficile and **Clostridium sordellii**)

IT Toxins
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(entero-, LT, UDP-glucose deficiency in mutant cell line and protection against **glucosyltransferase** toxins from Clostridium difficile and **Clostridium sordellii**)

IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(gene rho, UDP-glucose deficiency in mutant cell line and protection against **glucosyltransferase** toxins from Clostridium difficile and **Clostridium sordellii**)

IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(UDP-glucose deficiency in mutant cell line and protection against **glucosyltransferase** toxins from Clostridium difficile and **Clostridium sordellii**)

IT 133-89-1, UDP-glucose
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(UDP-glucose deficiency in mutant cell line and protection against **glucosyltransferase** toxins from Clostridium difficile and

Clostridium sordellii)
IT **9031-48-5, Glucosyltransferase**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(UDP-glucose deficiency in mutant cell line and protection against **glucosyltransferase** toxins from Clostridium difficile and **Clostridium sordellii**)
RN 9031-48-5 HCAPLUS
CN Glucosyltransferase (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

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     FILE 'WPIDS' ENTERED AT 07:54:47 ON 28 AUG 2000
L1           249 S  IMMUNOTOXIN?
L2             4 S (C OR CLOSTRIDIUM) (W) SORDELLII
L3          1247 S CLOSTRID?
L4             2 S L1 AND L3
L5          1171 S RAS
L6           117 S GLUCOSYLTRANSFERA? OR GLUCOSYL TRANFERAS?
L7            23 S TRANSLOCATION (2A) DOMAIN#
L8           157 S CATALYTIC (2A) (DOMAIN# OR ?PEPTIDE?)
L9             3 S L3 AND L5
L10            5 S L3 AND (L6 OR L7 OR L8)
L11            4 S L3 (S) (LT OR LETHAL TOXIN?)
L12           10 S L2 OR L4 OR L9 OR L10 OR L11

     FILE 'WPIDS' ENTERED AT 08:01:00 ON 28 AUG 2000

=> d .wp 1-10


L12  ANSWER 1 OF 10  WPIDS COPYRIGHT 2000   DERWENT INFORMATION LTD
AN   2000-376553 [32]   WPIDS
DNC  C2000-113955
TI   Novel composition, comprising superoxide dismutase linked by a cleavable
     linker to a neuronal cell targeting component useful for delivering
     superoxide dismutase to neuronal cells to treat ischemia.
DC   B04 D16
IN   HALLIS, B; SHONE, C C; SILMAN, N; SUTTON, J M
```

PA (MICR-N) MICROBIOLOGICAL RES AUTHORITY
CYC 90
PI WO 2000028041 A1 20000518 (200032)* EN 65p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL
TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
ADT WO 2000028041 A1 WO 1999-GB3699 19991105
PRAI GB 1998-24282 19981105
AB WO 200028041 A UPAB: 20000706
NOVELTY - Composition (I) comprising superoxide dismutase (SOD) linked by a cleavable linker to a neuronal cell targeting component (NCTC) with a domain that binds to a neuronal cell and a second domain that translocates the SOD of the composition into the neuronal cell, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) delivering SOD to a neuronal cell comprising administering (I);

(2) preparation of (I);

(3) a composition, for delivering a therapeutic agent to neuronal cells, comprising the therapeutic agent linked by a cleavable linker to a NCTC;

(4) a polypeptide (II) comprising a bacterial SOD or its derivative and sequence for targeting the polypeptide to a human mitochondria;

(5) a nucleotide (III) encoding (II);

(6) a vector (IV) comprising (III);

(7) preparation of (II); and

(8) a cell (V) comprising (III)/(IV).

ACTIVITY - Cerebroprotective; vasotropic; antiparkinsonian; nootropic.

A middle aged or elderly man diagnosed as suffering from stroke, was treated with an Mn-SOD construct within 6 hours of the stroke occurring. The construct (100 mg) was administered intravenously. Further doses were administered daily for 5-10 days. The ischemia/reperfusion damage was assessed by magnetic resonance imaging and was compared to a similarly affected untreated patient (control). Results showed that there were reduced levels of ischemia/reperfusion damage and relative improvements to muscle strength and co-ordination (MRC motor score) over a period of 12 months.

MECHANISM OF ACTION - Superoxide radical neutralizer.

USE - (I) is useful for treating neuronal diseases caused or augmented by oxidative stress (claimed) such as ischemic stroke, trauma, Parkinson's disease, Huntington's disease and motor neurone diseases.

ADVANTAGE - The bacterial SOD is less immunogenic and constructs of dimeric bacterial Mn-SOD is smaller in size compared to the human Mn-SOD.

DESCRIPTION OF DRAWING(S) - The diagram shows a recombinant Mn-SOD construct comprising a mitochondrial leader sequence, a Mn-SOD, a loop containing a unique protease site and which allows disulfide bridge formation, a **translocation domain**, and a neuronal targeting domain.

Dwg.3/5

L12 ANSWER 2 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 2000-224145 [19] WPIDS
DNC C2000-068326
TI New saponin derivatives with substituted triterpene aglycone core, used to potentiate antigens in vaccines against bacteria, viruses, protozoa and tumors.
DC A96 B01 B03 C02 D16
IN MARCIANI, D J; PRESS, J B
PA (MARC-I) MARCIANI D J; (PRES-I) PRESS J B
CYC 22
PI WO 2000009075 A2 20000224 (200019)* EN 99p
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP NO
AU 9955655 A 20000306 (200030)
ADT WO 2000009075 A2 WO 1999-US18635 19990813; AU 9955655 A AU 1999-55655 19990813
FDT AU 9955655 A Based on WO 200009075
PRAI US 1998-96691 19980814
AB WO 200009075 A UPAB: 20000419
NOVELTY - Saponin derivatives comprising a triterpene aglycone core substituted at positions 3 and 28 with a mono-or oligosaccharide are new.
DETAILED DESCRIPTION - A compound comprising a triterpene aglycone core wherein the core has a mono or oligo-saccharide covalently attached at position 3, a fucosyl residue covalently attached at position 28, wherein the fucosyl residue is optionally substituted with a mono- or oligo-saccharide and has a lipophilic group other than 3,5-dihydroxy-6-methyloctanoyl covalently attached to the 4 position, and a formyl or formylmethyl group covalently attached to the core at a position other than the 3 or 28 position.
An INDEPENDENT CLAIM is also included for a vaccine for human or veterinary use which comprises:
(a) one or more bacterial, viral, protozoal or tumor associated antigens; and
(b) one or more of the claimed saponin derivatives.
ACTIVITY - Immunopotentiators.
MECHANISM OF ACTION - None given.
USE - (I) are used as adjuvants in vaccine compositions used to vaccinate against bacteria, viruses, protozoa or tumors.
Dwg.0/2

L12 ANSWER 3 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 2000-072332 [06] WPIDS
DNC C2000-020614
TI New hybrid protein useful for inhibiting mast cell degranulation and treating allergic reactions.
DC B04 D16 J04
IN BIGALKE, H; FREVERT, J
PA (BIOT-N) BIOTECON-GES BIOTECHNOLOGISCHE
CYC 86
PI WO 9958571 A2 19991118 (200006)* DE 22p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9942605 A 19991129 (200018)

ADT WO 9958571 A2 WO 1999-EP3272 19990512; AU 9942605 A AU 1999-42605 19990512
FDT AU 9942605 A Based on WO 9958571
PRAI DE 1998-19821285 19980513
AB WO 9958571 A UPAB: 20000203
NOVELTY - A protein which binds to, or is absorbed by, mast cells or basophils is combined with a known protease (which cleaves proteins of the secretory apparatus of such cells) in a hybrid protein which is useful for inhibiting mast cell degranulation and treating allergic reactions.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (A) hybrid protein comprising: (a) a known protein which binds to (or is absorbed by) mast cells and/or basophils, in a known manner; and (b) a known protease which cleaves one or more proteins of the secretory apparatus of the mast cells or basophils. (B) hybrid protein comprising: (a) a protein which binds to (or is absorbed by) mast cells or basophils; and (b) a protease (especially a known protease) which cleaves one or more proteins of the secretory apparatus of the mast cells or basophils. Component (a) is selected from (i) IgE, (ii) IgE fragments (especially an IgE-Fc fragment), (iii) antibodies against IgE receptors of mast cells and/or basophils, (iv) fragments of antibodies against IgE receptors of mast cells and/or basophils (especially an Fab fragment), (v) antibodies against the mast cell-specific potassium channel, and (vi) inactive (though binding) MCD peptide. (C) hybrid protein comprising: (a) a protein (especially a known protein) which binds to (or is absorbed by) mast cells and/or basophils; and (b) a protease which cleaves one or more proteins of the secretion apparatus of the mast cells or basophils. The protease is selected from (i) the light chain of a **Clostridum** botulinum toxin (especially type A, B, Cl, D, E, F or G), (ii) the light chain of Tetanus toxin, (iii) catalytically active fragments of the light chains described in (i) or (ii), (iv) IgA protease from Neisseria gonorrhea or (v) **catalytic domains** of IgA protease from Neisseria gonorrhea.
ACTIVITY - Antiallergic.
USE - The hybrid proteins inhibit mast cell degranulation, and may be used in treatment or prevention of allergic reactions.
Dwg.0/0

L12 ANSWER 4 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1999-590957 [50] WPIDS
DNN N1999-435918 DNC C1999-172476
TI A new mutant of Escherichia coli holotoxin useful as an adjuvant.
DC B04 D16 S03
IN CLEMENTS, J D
PA (TULA) TULANE EDUCATIONAL FUND
CYC 86
PI WO 9947167 A1 19990923 (199950)* EN 27p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU

LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR
TT UA UG UZ VN YU ZA ZW
AU 9930893 A 19991011 (200008)
US 6033673 A 20000307 (200019)
ADT WO 9947167 A1 WO 1999-US5623 19990317; AU 9930893 A AU 1999-30893 19990317; US 6033673 A US 1998-44064 19980318
FDT AU 9930893 A Based on WO 9947167
PRAI US 1998-44064 19980318
AB WO 9947167 A UPAB: 19991201
NOVELTY - A mutant Escherichia coli heat-labile enterotoxin holotoxin (LT), having mutations at positions 192 and 211, is new.
DETAILED DESCRIPTION - The mutant E. coli LT, has Gly substituted for Arg at position 192, and Ala substituted for Leu at position 211, and immunological adjuvant activity. The holotoxin is substantially less toxic than native E. coli LT as measured in the Y-1 adrenal cell assay or patent mouse assay, and less toxic than isolated LT(R192G) as measured in the patent mouse assay. INDEPENDENT CLAIMS are also included for the following: (i) a preparation (I) comprising an antigen and mutant LT; (ii) a kit useful for producing a protective immune response in a host to a pathogen comprising (I); (iii) creating or sustaining a protective or adaptive response, or inducing a protective immune response to an antigen in a host, comprising orally administering (I); (iv) inducing a protective immune response against an enterotoxic bacterial organism, particularly those that express a cholera-like toxin, most particularly Escherichia or Vibrio species, comprising administering mutant LT as a component of a vaccine.
ACTIVITY - Stimulatory; LT stimulates the immune response.
MECHANISM OF ACTION - Catalyst.
USE - The mutant LT is used as an adjuvant in conjunction with an antigen to create, induce or sustain an immune response (claimed). The mutant LT is also used in a vaccine to induce a protective immune response against an enterotoxic bacteria (claimed).
ADVANTAGE - The mutant LT has increased adjuvant activity for induction of serum IgG and mucosal IgA against measles virus compared with prior art native LT, LT-B and LT(E112K). The mutant LT is also less toxic than native LT.
Dwg.0/8

L12 ANSWER 5 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1999-509323 [43] WPIDS
DNC C1999-149044
TI New fragment of the **lethal toxin** from **Clostridium** bacterium, useful for treating cancer.
DC B04 D16
IN AKTORIES, K; HOFMANN, F
PA (UYFR-N) UNIV FREIBURG ALBERT-LUDWIGS
CYC 1
PI DE 19802569 A1 19990909 (199943)* 14p
ADT DE 19802569 A1 DE 1998-19802569 19980123
PRAI DE 1998-19802569 19980123
AB DE 19802569 A UPAB: 19991020

NOVELTY - A fragment (I) of the **lethal toxin** (**LT**) of **Clostridium** is new and has at least 80% homology to the 546 amino acid (aa) sequence (1) given in the specification.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for an **immunotoxin** (II), consisting of (I) and a cell-binding component (III).

ACTIVITY - Antitumor.

MECHANISM OF ACTION - (I) is a glucosyl transferase that glycosylates, and thus inactivates, GTP(guanine triphosphate)ases, particularly **Ras** (an oncogenic product overexpressed in many tumors), resulting in inhibition of epidermal growth factor-stimulated MAP-kinase signaling pathways. **Ras** protein (1 mu g) and (I) (1 nM) were incubated in the presence of radiolabeled UDP (uridine diphosphate)-glucose (10 mu M), for various times, then labeled proteins separated by electrophoresis and quantitated by phosphor imaging. (I) was about 20% more active in glycosylation of **Ras** than the holoenzyme.

USE - (I), particularly in the form of **immunotoxins**, are used as cell-specific toxins, particularly for treating cancer.

ADVANTAGE - When included in **immunotoxins**, (I) can be targeted to selected cells. Compared with the complete LT, (I) is smaller, so enters cells more easily, resulting in greater toxicity in the cytosol, is less likely to induce formation of (neutralizing) antibodies, and is more active than the holotoxin.

Dwg.0/1

L12 ANSWER 6 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1999-182094 [16] WPIDS
DNC C1999-053308
TI Monoclonal antibodies specific for **Clostridium** difficile toxins - especially humanised antibodies for treating pseudomembranous colitis.
DC B04 D16
IN MOOS, M; VON EICHEL-STREIBER, C
PA (VEIC-I) VON EICHEL-STREIBER C
CYC 83
PI DE 19739685 A1 19990311 (199916)* 14p
WO 9912971 A2 19990318 (199918) DE
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 9897426 A 19990329 (199932)
EP 994904 A2 20000426 (200025) DE
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
ADT DE 19739685 A1 DE 1997-19739685 19970910; WO 9912971 A2 WO 1998-EP5759 19980910; AU 9897426 A AU 1998-97426 19980910; EP 994904 A2 EP 1998-951374 19980910, WO 1998-EP5759 19980910
FDT AU 9897426 A Based on WO 9912971; EP 994904 A2 Based on WO 9912971
PRAI DE 1997-19739685 19970910
AB DE 19739685 A UPAB: 19990424
A monoclonal antibody that is directed against a **Clostridium**

difficile toxin and recognises and neutralises an epitope in the ligand **domain, translocation domain** or **catalytic domain** of the toxin is new.

USE - Humanised antibodies as above, especially when expressed in plants, can be used for immunotherapy of diseases caused by **Clostridium** difficile enterotoxin (toxin A) or cytotoxin (toxin B), especially pseudomembranous colitis.

Dwg.0/0

L12 ANSWER 7 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1997-402313 [37] WPIDS
DNC C1997-129748
TI Use of **Clostridium sordellii lethal toxin** - for inactivating **Ras** by glucosylation, used for treating conditions such as cancer, particularly pancreatic or colon cancer.
DC B04 D16
IN BOQUET, P; THELESTAM, M; VON EICHELSTREIBER, C; VON EICHEL-STREIBER, C
PA (BOEF) BOEHRINGER MANNHEIM GMBH; (ASTA) ASTA MEDICA AG
CYC 75
PI WO 9727871 A1 19970807 (199737)* EN 45p
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN
AU 9715982 A 19970822 (199801)
EP 877622 A1 19981118 (199850) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
ADT WO 9727871 A1 WO 1997-EP426 19970131; AU 9715982 A AU 1997-15982 19970131,
WO 1997-EP426 19970131; EP 877622 A1 EP 1997-902278 19970131, WO 1997-EP426 19970131
FDT AU 9715982 A Based on WO 9727871; EP 877622 A1 Based on WO 9727871
PRAI EP 1996-101469 19960202
AB WO 9727871 A UPAB: 19970915
An **immunotoxin** (A) comprises a first, second and third part, connected by covalent bonds and a pharmaceutically acceptable carrier: (a) the first part includes a target cell specific binding domain, which is able to cause the **LT** (**lethal toxin**) **immunotoxin** of **Clostridium sordellii** (CS) to bind to the patient's cell; (b) the second part includes a **translocation domain** of a protein capable of translocating the third part across the cytoplasmic membrane of the cell; and (c) the third part includes a polypeptide with the toxic activity of the **catalytic domain** of **LT** from CS.

A composition for the treatment of a pathological disorder associated with the activation of **Ras** proto-oncoproteins comprising (A) and a pharmaceutically acceptable carrier is also claimed.

USE - The CS LT can inactivate **Ras** by glucosylation of **Ras** threonine 35. The products can be used for treating cancers, particularly pancrease or colon cancer.

Dwg.0/7

L12 ANSWER 8 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 1996-308052 [31] WPIDS
DNC C1996-098383
TI Synergistic vaccine-based medicament for ruminants - contg. vitamin-B12 to combat cobalt deficiency, improve propionic acid metabolism and boost response to vaccine.
DC B02 B04 C02 C06 D16
IN LEECH, W F; MCLAREN, D G
PA (BOMA-N) BOMAC LAB LTD
CYC 1
PI NZ 241920 A 19960528 (199631)* 16p
ADT NZ 241920 A NZ 1992-241920 19920311
PRAI NZ 1992-241920 19920311
AB NZ 241920 A UPAB: 19960808
Synergistic medicament comprises:(A) vitamin B12 or a physiologically effective equiv. deriv., and(B) a vaccine.

Also claimed are:(a) a pack comprising 2 containers, 1 contg. (A) and 1 contg. (B), where (A) and (B) are present in amts. which are synergistically effective on simultaneous admin.;(b) a method of treating a ruminant by simultaneously injecting (A) and (B) to provide the benefits of each component while reducing the adverse effects of the antigenic challenge, or by injection of the medicament contg. (A) and (B);(c) a stable injectable compsn. for a ruminant which comprises (A) and (B) in an injectable carrier liq. and having pH 6, and(d) the use of the compsn. as in (d) by injection in a ruminant.

USE - The medicaments are useful in sheep, lambs, goats and calves. Vaccine (B) is specifically against gas gangrene (Clostridium perfringens A), lamb dysentery (C. perfringens D), malignant oedema (C. septicum), blackleg (C. chauvoei), tetanus (C. tetani), black disease (C. novyi B), haemoglobinuria (C. haemolyticum), sordelli infections (**C. sordellii**), caseous lymphadenitis (Corynebacterium ovis), haemorrhagic septicaemia (Pasteurella multocida and P. haemolytica), leptospirosis (Leptospira sp.), salmonellosis (Salmonella sp.) and/or foot-rot (Fusiformis nodosus) (all claimed). (A) combats cobalt deficiency (which interferes with utilisation of the major energy source propionic acid) and thus improves the well-being of the animal, e.g. to improve wt. gain, growth and (where applicable) wool quality. Doses of (A) and (B) are such as to allow injection of (A) to balance the effect of antigenic challenge by (B), where the injectable prepn. complies with the British Veterinary Codex for the antigenic units (claimed). Typically the C. perfringens B antitoxin dose (s.c.) is 15000 U for sheep or 6000 U for lambs.

ADVANTAGE - (A) enhances animal metabolism and acts as a general immunological booster, allowing better response to the vaccine due to improved health. Response to the vaccine is synergistically improved and the immediate adverse effects of antigenic challenge are minimised. Cobalt is available to the animal for an extended period, the vaccines have acceptable stability, (A) does not affect the antigenic potency of (B) and (B) does not affect the (A) concn.
Dwg.0/0

L12 ANSWER 9 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1994-332824 [41] WPIDS
DNC C1994-151344
TI Multicomponent clostridial vaccine compsns. - contg. a rapidly dispersed component, e.g. saponin, as adjuvant.
DC B04 D16
IN ROBERTS, D S
PA (PFIZ) PFIZER INC; (SMIK) SMITHKLINE BEECHAM CORP; (ROBE-I) ROBERTS D S
CYC 21
PI WO 9422476 A1 19941013 (199441)* EN 25p
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU CA JP US
AU 9464939 A 19941024 (199505)
EP 692974 A1 19960124 (199609) EN
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
JP 08510206 W 19961029 (199705) 26p
AU 689772 B 19980409 (199827)
US 6083512 A 20000704 (200036)
ADT WO 9422476 A1 WO 1994-US3395 19940329; AU 9464939 A AU 1994-64939 19940329; EP 692974 A1 EP 1994-912333 19940329, WO 1994-US3395 19940329; JP 08510206 W JP 1994-522287 19940329, WO 1994-US3395 19940329; AU 689772 B AU 1994-64939 19940329; US 6083512 A Cont of US 1993-38428 19930329, Cont of WO 1994-US3395 19940329, US 1995-536970 19950929
FDT AU 9464939 A Based on WO 9422476; EP 692974 A1 Based on WO 9422476; JP 08510206 W Based on WO 9422476; AU 689772 B Previous Publ. AU 9464939, Based on WO 9422476
PRAI US 1993-38428 19930329; US 1995-536970 19950929
AB WO 9422476 A UPAB: 19941206
Multicomponent clostridial vaccine compsn. comprises two or more clostridial immunogens and a rapidly dispersed adjuvant.

The adjuvant is saponin. The clostridial immunogens comprise two or more clostridial bacterins or toxoids, esp. deriv. from Clostridium perfringens, Clostridium septicum, Clostridium tetani, Clostridium chauvoei, Clostridium novyi, **Clostridium sordellii**, Clostridium haemolyticum, Clostridium botulism, Clostridium perfringens, and serotypes of these. The vaccine is administered by intramuscular or subcutaneous injection.

USE/ADVANTAGE - The vaccines can be used to protect mammals, esp. cattle, against red water disease, big head, blackleg, enteroxemias, infections nectotic hepatitis, malignant oedema, botulism, tetanus, etc. The vaccines are safe and non-toxic.
Dwg.0/0

L12 ANSWER 10 OF 10 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1972-02392T [02] WPIDS
TI Tetanus antitoxin - from the milk system of cows by hyperimmunisation with
tetanus toxoid and toxin.
DC B04 D16
PA (IMMN) IMMUNE MILK CO
CYC 1
PI US 3626057 A (197202)*
PRAI US 1965-505023 19651024; US 1968-747106 19680724; US 1969-860815 19690924
AB US 3626057 A UPAB: 19930000
Antitoxin is prepd. by (a) injecting **Clostridium** tetani (strain No. 9441 American Type Culture) into a sealed, sterile veal infusion

broth, incubating at 37 degrees C for 12 days, standardising the sterility tested purified toxin as to MLD and **Lt** dose, and converting a portion of the toxin to toxoid by heating at 60 degrees C for 1 hr. The toxoid is injected at 4 day intervals with doses increasing from 15 cc to 80 cc over 104 days. The toxin having 1:200,000 MLD/cc, is then injected at 1 week intervals with doses increasing from 10 cc to 100 cc over 8 weeks. The milk is then collected and the antitoxin extracted by ammonium sulphate fractionation.

```
=> fil medline

FILE 'MEDLINE' ENTERED AT 08:12:57 ON 28 AUG 2000

 FILE LAST UPDATED: 24 AUG 2000 (20000824/UP).  FILE COVERS 1960 TO DATE.

 MEDLINE has been reloaded to reflect the annual MeSH changes made by
 the National Library of Medicine for 2000. Enter HELP RLOAD for details.

 The OLDMEDLINE file segment now contains data from 1958 through 1965.
 Enter HELP CONTENT for details.

 Left, right, and simultaneous left and right truncation are available in the
 Basic Index.  See HELP SFIELDS for details.

 THIS FILE CONTAINS CAS REGISTRY NUMBERS FOR EASY AND ACCURATE
 SUBSTANCE IDENTIFICATION.

=> d his


     (FILE 'MEDLINE' ENTERED AT 08:01:37 ON 28 AUG 2000)
                DEL HIS Y
L1           6083 S CLOSTRIDIUM/CT
L2          18461 S RAS
L3           9965 S GLYCOSYLATION/CT
L4           8680 S BACTERIAL TOXINS/CT
L5             14 S L1 AND L2
L6            217 S SORDELLII
L7              9 S L5 AND L6
L8            145 S L1 AND L6
L9             43 S L8 AND (L2 OR L3 OR L4)
L10             9 S L8 AND (L2 OR L3)
L11           431 S L1 AND L4
L12             1 S L11 AND (TRANSLOCATION DOMAIN)
L13            10 S L7 OR L10 OR L12

     FILE 'MEDLINE' ENTERED AT 08:12:57 ON 28 AUG 2000

=> d .med 1-10


L13  ANSWER 1 OF 10  MEDLINE
AN   1999253957     MEDLINE
DN   99253957
TI   G-protein-stimulated phospholipase D activity is inhibited by lethal
toxin
     from Clostridium sordellii in HL-60 cells.
AU   El Hadj N B; Popoff M R; Marvaud J C; Payrastre B; Boquet P; Geny B
CS   INSERM U332, ICGM, 22 rue Mechain, 75014 Paris, France.
SO   JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 May 14) 274 (20) 14021-31.
     Journal code: HIV. ISSN: 0021-9258.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals; Cancer Journals
```

EM 199908
AB Lethal toxin (LT) from Clostridium **sordellii** has been shown in HeLa cells to glucosylate and inactivate **Ras** and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that LT treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with LT. A similar dose response to the toxin was found when PLD activity was stimulated by phorbol 12-myristate 13-acetate via the protein kinase C pathway. The toxin effect on actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota toxin from Clostridium perfringens E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease observed in phosphatidylinositol 4,5-bisphosphate (PtdIns(4, 5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in LT-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. LT was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, Rac, and RalA, inactivated by LT and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as LT toxin (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, LT treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosol prepared from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase Calpha was decreased after LT treatment. We conclude that in HL-60 cells, lethal toxin from C. **sordellii**, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications observed in HL-60 cells.
CT Check Tags: Human; Support, Non-U.S. Gov't
ras Proteins: ME, metabolism
Androstadienes: PD, pharmacology
*Bacterial Toxins: PD, pharmacology
Clostridium
Cytosol: ME, metabolism
Enzyme Inhibitors: PD, pharmacology
*Glucosyltransferases: ME, metabolism
Glycosylation

Guanosine 5'-O-(3-Thiotriphosphate): PD, pharmacology
GTP Phosphohydrolases: ME, metabolism
*GTP-Binding Proteins: ME, metabolism
HL-60 Cells
*Phospholipase D: ME, metabolism
1-Phosphatidylinositol 3-Kinase: ME, metabolism
1-Phosphatidylinositol 4-Kinase: ME, metabolism

L13 ANSWER 2 OF 10 MEDLINE
AN 1999102800 MEDLINE
DN 99102800
TI Inhibition of small G proteins by clostridium **sordellii** lethal toxin activates cdc2 and MAP kinase in Xenopus oocytes.
AU Rime H; Talbi N; Popoff M R; Suziedelis K; Jessus C; Ozon R
CS INRA/ESA-CNRS 7080, Universite Pierre et Marie Curie, 4 place Jussieu, 75252 Paris Cedex 05, France.
SO DEVELOPMENTAL BIOLOGY, (1998 Dec 15) 204 (2) 592-602.
Journal code: E7T. ISSN: 0012-1606.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199904
AB The lethal toxin (LT) from Clostridium **sordellii** is a glucosyltransferase that modifies and inhibits small G proteins of the **Ras** family, **Ras** and Rap, as well as Rac proteins. LT induces cdc2 kinase activation and germinal vesicle breakdown (GVBD) when microinjected into full-grown Xenopus oocytes. Toxin B from Clostridium difficile, that glucosylates and inactivates Rac proteins, does not induce cdc2 activation, indicating that proteins of the **Ras** family, **Ras** and/or Rap, negatively regulate cdc2 kinase activation in Xenopus oocyte. In oocyte extracts, LT catalyzes the incorporation of [14C]glucose into a group of proteins of 23 kDa and into one protein of 27 kDa. The 23-kDa proteins are recognized by anti-Rap1 and anti-Rap2 antibodies, whereas the 27-kDa protein is recognized by several anti-**Ras** antibodies and probably corresponds to K-**Ras**. Microinjection of LT into oocytes together with UDP-[14C]glucose results in a glucosylation pattern similar to the in vitro glucosylation, indicating that the 23- and 27-kDa proteins are in vivo substrates of LT. In vivo time-course analysis reveals that the 27-kDa protein glucosylation is completed within 2 h, well before cdc2 kinase activation, whereas the 23-kDa proteins are partially glucosylated at GVBD. This observation suggests that the 27-kDa **Ras** protein could be the in vivo target of LT allowing cdc2 kinase activation. Interestingly, inactivation of **Ras** proteins does not prevent the phosphorylation of c-Raf1 and the activation of MAP kinase that occurs normally around GVBD. Copyright 1998 Academic Press.
CT Check Tags: Animal; Female; Support, Non-U.S. Gov't
*Bacterial Toxins: TO, toxicity
*Ca(2+)-Calmodulin Dependent Protein Kinase: ME, metabolism
Clostridium
Enzyme Activation: DE, drug effects
*GTP-Binding Proteins: AI, antagonists & inhibitors
Oocytes: DE, drug effects

*Oocytes: ME, metabolism
*Protein p34cdc2: ME, metabolism
*Signal Transduction: DE, drug effects
Xenopus

L13 ANSWER 3 OF 10 MEDLINE
AN 1998344048 MEDLINE
DN 98344048
TI A common motif of eukaryotic glycosyltransferases is essential for the enzyme activity of large clostridial cytotoxins.
AU Busch C; Hofmann F; Selzer J; Munro S; Jeckel D; Aktories K
CS Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Hermann-Herder-Str. 5, D-79104 Freiburg, Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jul 31) 273 (31) 19566-72.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199811
AB A fragment of the N-terminal 546 amino acid residues of Clostridium **sordellii** lethal toxin possesses full enzyme activity and glucosylates Rho and **Ras** GTPases in vitro. Here we identified several amino acid residues in C. **sordellii** lethal toxin that are essential for the enzyme activity of the active toxin fragment. Exchange of aspartic acid at position 286 or 288 with alanine or asparagine decreased glucosyltransferase activity by about 5000-fold and completely blocked glucohydrolase activity. No enzyme activity was detected with the double mutant D286A/D288A. Whereas the wild-type fragment of C. **sordellii** lethal toxin was labeled by azido-UDP-glucose after UV irradiation, mutation of the DXD motif prevented radiolabeling. At high concentrations (10 mM) of manganese ions, the transferase activities of the D286A and D288A mutants but not that of wild-type fragment were increased by about 20-fold. The exchange of Asp270 and Arg273 reduced glucosyltransferase activity by about 200-fold and blocked glucohydrolase activity. The data indicate that the DXD motif, which is highly conserved in all large clostridial cytotoxins and also in a large number of glycosyltransferases, is functionally essential for the enzyme activity of the toxins and may participate in coordination of the divalent cation and/or in the binding of UDP-glucose.
CT Check Tags: Human; Support, Non-U.S. Gov't
Amino Acid Sequence
*Bacterial Toxins: CH, chemistry
***Clostridium: EN, enzymology**
*Glucosyltransferases: CH, chemistry
Glucosyltransferases: ME, metabolism
Glycosylation
GTP-Binding Proteins: GE, genetics
GTP-Binding Proteins: ME, metabolism
Hela Cells: DE, drug effects
Hydrolases: ME, metabolism
Kinetics
Manganese: PD, pharmacology
Molecular Sequence Data
Mutagenesis, Site-Directed: GE, genetics

Peptide Fragments: ME, metabolism
Photoaffinity Labels: ME, metabolism
Recombinant Proteins: ME, metabolism
Sequence Alignment
Sequence Analysis
Substrate Specificity
Uridine Diphosphate Glucose: ME, metabolism

L13 ANSWER 4 OF 10 MEDLINE
AN 1998298120 MEDLINE
DN 98298120
TI Functional consequences of monoglucosylation of Ha-**Ras** at effector domain amino acid threonine 35.
AU Herrmann C; Ahmadian M R; Hofmann F; Just I
CS Max-Planck-Institut fur Molekulare Physiologie, Rheinlanddamm 201, D-44139
Dortmund, Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 26) 273 (26) 16134-9.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199810
AB Monoglucosylation of low molecular mass GTPases is an important post-translational modification by which microbes interfere with eukaryotic cell signaling. Ha-**Ras** is monoglucosylated at effector domain amino acid threonine 35 by Clostridium **sordellii** lethal toxin, resulting in a blockade of the downstream mitogen-activated protein kinase cascade. To understand the molecular consequences of this modification, effects of glucosylation on each step of the GTPase cycle of
Ras were analyzed. Whereas nucleotide binding was not significantly altered, intrinsic GTPase activity was markedly decreased, and GTPase stimulation by the GTPase-activating protein p120(GAP) and neurofibromin NF-1 was completely blocked, caused by failure to bind to glucosylated **Ras**. Guanine nucleotide exchange factor (Cdc25)-catalyzed GTP loading was decreased, but not completely inhibited.
A dominant-negative property of modified **Ras** to sequester exchange factor was not detectable. However, the crucial step in downstream signaling, **Ras**-effector coupling, was completely blocked. The Kd for the interaction between **Ras**.GTP and the **Ras**-binding domain of Raf was 15 nM, whereas glucosylation increased the Kd to >1 mM. Because the affinity of **Ras**.GDP for Raf (Kd = 22 &mgr;M) is too low to allow functional interaction, a glucose
moiety at threonine 35 of **Ras** seems to block completely the interaction with Raf. The net effect of lethal toxin-catalyzed glucosylation of **Ras** is the complete blockade of **Ras** downstream signaling.
CT Check Tags: Support, Non-U.S. Gov't
***ras Proteins: ME, metabolism**
Bacterial Toxins: ME, metabolism
Catalysis
Clostridium
DNA-Binding Proteins: ME, metabolism

Glucosyltransferases: ME, metabolism
Glycosylation
GTP Phosphohydrolases: ME, metabolism
Kinetics
Structure-Activity Relationship
*Threonine: ME, metabolism
Uridine Diphosphate Glucose: ME, metabolism

L13 ANSWER 5 OF 10 MEDLINE
AN 1998184846 MEDLINE
DN 98184846
TI Specific inhibition of phorbol ester-stimulated phospholipase D by Clostridium **sordellii** lethal toxin and Clostridium difficile toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases.
AU Schmidt M; Voss M; Thiel M; Bauer B; Grannass A; Tapp E; Cool R H; de Gunzburg J; von Eichel-Streiber C; Jakobs K H
CS Institut fur Pharmakologie, Universitatsklinikum Essen, D-45122 Essen, Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 27) 273 (13) 7413-22. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199806
AB Activation of m3 muscarinic acetylcholine receptor (mAChR), stably expressed in human embryonic kidney (HEK)-293 cells, leads to phospholipase D (PLD) stimulation, a process apparently involving Rho GTPases, as shown by studies with Clostridium botulinum C3 exoenzyme and Clostridium difficile toxin B (TcdB). Direct activation of protein kinase C (PKC) by phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), also induces PLD stimulation, which is additive to the mAChR action and which is only poorly sensitive to inactivation of Rho proteins by TcdB. To study whether **Ras**-like GTPases are involved in PLD regulation, we studied the effects of the TcdB variant TcdB-1470 and Clostridium **sordellii** lethal toxin (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by mAChR or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time- and concentration-dependent manner, without alteration in immunologically detectable PKC isozyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addition of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addition of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addition of recombinant Ral proteins (RalA

and RalB), glucosylation substrates for TscL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddition of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
*Bacterial Toxins: PD, pharmacology
Cell Line
Clostridium
Clostridium difficile
Enzyme Activation: DE, drug effects
*Glucosyltransferases: ME, metabolism
*GTP Phosphohydrolases: ME, metabolism
*GTP-Binding Proteins: ME, metabolism
Mice
*Phospholipase D: ME, metabolism
Protein Kinase C: ME, metabolism
Receptors, Muscarinic: ME, metabolism
Signal Transduction
*Tetradecanoylphorbol Acetate: PD, pharmacology
3T3 Cells

L13 ANSWER 6 OF 10 MEDLINE
AN 97151733 MEDLINE
DN 97151733
TI Immunological and functional comparison between Clostridium perfringens iota toxin, C. spiroforme toxin, and anthrax toxins.
AU Perelle S; Scalzo S; Kochi S; Mock M; Popoff M R
CS Unite des Toxines Microbiennes, CNRS URA1858, Institut Pasteur, Paris, France.
SO FEMS MICROBIOLOGY LETTERS, (1997 Jan 1) 146 (1) 117-21.
Journal code: FML. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199705
EW 19970503
AB Clostridium perfringens iota and C. spiroforme toxins consist of two separate proteins. One is the binding component and the other the enzymatic component. The two toxins secreted by Bacillus anthracis are composed of binary combinations of three proteins: protective antigen, lethal factor, and edema factor. As shown by Western blotting and ELISA, the binding component of anthrax toxin shares common epitopes with that of iota toxin and C. spiroforme toxin which are closely related immunologically. However, no functional complementation was observed between iota toxin and anthrax toxin components. The binding components can form toxins active on macrophages only in combination with their respective enzymatic components. Agents which prevent acidification of endosomes do not have the same effects on anthrax toxin activity as they do on iota and C. spiroforme toxins. Therefore, the mechanisms of entry

into the cells are presumably different. Since the binding components of anthrax toxins and iota toxin share a conserved putative **translocation domain**, these binding components could have a common mode of insertion into the cell membranes.

CT Check Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't
Antigens, Bacterial: CH, chemistry
*Bacillus anthracis: IM, immunology
Bacterial Toxins: CH, chemistry
***Bacterial Toxins: IM, immunology**
Bacterial Toxins: TO, toxicity
Binding Sites
Cell Membrane: DE, drug effects
***Clostridium: IM, immunology**
*Clostridium perfringens: IM, immunology
Immunochemistry
Molecular Structure

L13 ANSWER 7 OF 10 MEDLINE
AN 97127410 MEDLINE
DN 97127410
TI Difference in protein substrate specificity between hemorrhagic toxin and lethal toxin from Clostridium **sordellii**.
AU Genth H; Hofmann F; Selzer J; Rex G; Aktories K; Just I
CS Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Germany.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1996 Dec 13) 229 (2) 370-4.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199703
EW 19970304
AB The hemorrhagic toxin (HT) from Clostridium **sordellii** is pharmacologically related to Clostridium difficile toxins A and B and Clostridium **sordellii** lethal toxin which have been recently identified as mono-glucosyl-transferases. Here we report that HT, which is coexpressed with lethal toxin, is also a glucosyltransferase. Whereas lethal toxin glucosylates the Rho subfamily proteins Rac and Cdc42 and the **Ras** subfamily proteins H-**Ras** and Rap, the substrate specificity of HT is strictly confined to the Rho subfamily proteins Rho, Rac and Cdc42. Comparable to lethal toxin, transferase activity of HT is stimulated by Mn^{2+}. Acceptor amino acid in Rho was identified by mutagenesis as threonine-37. C. **sordellii** HT is a novel member of the family of clostridial mono-glucosyl-transferases, a family which modifies the Rho and **Ras** GTPases.
CT Check Tags: Animal; Support, Non-U.S. Gov't
*Bacterial Toxins: ME, metabolism
Catalysis
Clostridium: EN, enzymology
***Clostridium: ME, metabolism**
Glycosylation
Glycosyltransferases: ME, metabolism

Mice
Substrate Specificity
3T3 Cells

L13 ANSWER 8 OF 10 MEDLINE
AN 97011096 MEDLINE
DN 97011096
TI The **ras**-related protein Ral is monoglucosylated by Clostridium **sordellii** lethal toxin.
AU Hofmann F; Rex G; Aktories K; Just I
CS Institut fur Pharmakologie and Toxikologie, Albert-Ludwigs-Universitat Freiburg, Germany.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1996 Oct 3) 227 (1) 77-81.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199701
AB Clostridium **sordellii** lethal toxin (LT), a cytotoxin which causes preferential destruction of the actin cytoskeleton, has been recently identified as glucosyltransferase to modify the low molecular mass GTPases Rac, **Ras** and Rap. We report here on LT produced by C. **sordellii** strain 6018 which glucosylates in addition to Rac, **Ras** and Rap the Ral protein. LT from strain VPI9048 however does not glucosylate Ral. Besides recombinant Ral, cellular Ral is also substrate. In the GDP-bound form, Ral is a superior substrate to the GTP form. Acceptor amino acid for glucose is threonine-46 which is equivalent to threonine-35 in H-**Ras** located in the effector region. The Ral-glucosylating toxin is a novel isoform of **Ras**-modifying clostridial cytotoxins.
CT Check Tags: Animal; Support, Non-U.S. Gov't
*Bacterial Toxins: ME, metabolism
Binding Sites
***Clostridium: ME, metabolism**
Glycosylation
Guanosine Triphosphate: ME, metabolism
GTP-Binding Proteins: GE, genetics
*GTP-Binding Proteins: ME, metabolism
Molecular Weight
Mutagenesis, Site-Directed
Rats

L13 ANSWER 9 OF 10 MEDLINE
AN 96215317 MEDLINE
DN 96215317
TI **Ras**, Rap, and Rac small GTP-binding proteins are targets for Clostridium **sordellii** lethal toxin glucosylation.
AU Popoff M R; Chaves-Olarte E; Lemichez E; von Eichel-Streiber C; Thelestam M; Chardin P; Cussac D; Antonny B; Chavrier P; Flatau G; Giry M; de Gunzburg J; Boquet P
CS Institut Pasteur, Unite des Toxines Microbiennes, 75724 Paris, Cedex 15, France.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 26) 271 (17) 10217-24.
Journal code: HIV. ISSN: 0021-9258.
CY United States

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199608
AB Lethal toxin (LT) from Clostridium **sordellii** is one of the high molecular mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that LT is a glucosyltransferase that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. LT glucosylates **Ras**, Rap, and Rac. In **Ras**, threonine at position 35 was identified as the target amino acid glucosylated by LT.

Other related members of the **Ras** GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by LT. Incubation of serum-starved Swiss 3T3 cells with LT prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the toxin blocks **Ras** function in vivo. We also demonstrate that LT acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphology. LT is thus a powerful tool to inhibit **Ras** function in vivo.

CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
Actins: CH, chemistry
Amino Acid Sequence
*Bacterial Toxins: ME, metabolism
Bacterial Toxins: TO, toxicity
Ca(2+)-Calmodulin Dependent Protein Kinase: ME, metabolism
***Clostridium: PY, pathogenicity**
Epidermal Growth Factor: PD, pharmacology
Glucose: ME, metabolism
*Glucosyltransferases: ME, metabolism
Guanosine Triphosphate: ME, metabolism
GTP Phosphohydrolases: ME, metabolism
*GTP-Binding Proteins: ME, metabolism
Hela Cells: DE, drug effects
Hela Cells: UL, ultrastructure
Kinetics
Mice
Microfilaments: UL, ultrastructure
Molecular Sequence Data
***Proto-Oncogene Protein p21(ras): ME, metabolism**
Threonine: ME, metabolism
Uridine Diphosphate Glucose: ME, metabolism
3T3 Cells

L13 ANSWER 10 OF 10 MEDLINE
AN 96215306 MEDLINE
DN 96215306
TI Inactivation of **Ras** by Clostridium **sordellii** lethal toxin-catalyzed glucosylation.
AU Just I; Selzer J; Hofmann F; Green G A; Aktories K
CS Institut fur Pharmakologie und Toxikologie der Universitat Freiburg, Hermann-Herder-Strasse 5, D-79104 Freiburg, Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 26) 271 (17) 10149-53. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)

LA English
FS Priority Journals; Cancer Journals
EM 199608
AB The lethal toxin (LT) from Clostridium **sordellii** belongs to the family of large clostridial cytotoxins causing morphological alterations in cultured cell lines accompanied by destruction of the actin cytoskeleton. C. **sordellii** LT exhibits 90% homology to Clostridium difficile toxin B, which has been recently identified as a monoglucosyltransferase (Just, I., Selzer, J., Wilm, M., von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) Nature 375, 500-503). We report here that LT too is a glucosyltransferase, which uses UDP-glucose as cosubstrate to modify low molecular mass GTPases. LT selectively modifies Rac and **Ras**, whereas the substrate specificity of toxin B is confined to the Rho subfamily proteins Rho, Rac, and Cdc42, which participate in the regulation of the actin cytoskeleton. In Rac, both toxin B and LT share the same acceptor amino acid, threonine 35. Glucosylation of **Ras** by LT results in inhibition of the epidermal growth factor-stimulated p42/p44 MAP-kinase signal pathway. LT is the first bacterial toxin to inactivate **Ras** in intact cells.
CT Check Tags: Animal; Support, Non-U.S. Gov't
*Bacterial Toxins: ME, metabolism
Ca(2+)-Calmodulin Dependent Protein Kinase: ME, metabolism
Cations, Divalent
***Clostridium*: ME, metabolism**
*Glucosyltransferases: ME, metabolism
Guanosine Diphosphate: ME, metabolism
Guanosine Triphosphate: ME, metabolism
GTP-Binding Proteins: ME, metabolism
Mice
***Proto-Oncogene Protein p21(ras): ME, metabolism**
Rats
3T3 Cells

```
=> d que

L1          6083 SEA FILE=MEDLINE ABB=ON  CLOSTRIDIUM/CT
L2         18461 SEA FILE=MEDLINE ABB=ON  RAS
L3          9965 SEA FILE=MEDLINE ABB=ON  GLYCOSYLATION/CT
L4          8680 SEA FILE=MEDLINE ABB=ON  BACTERIAL TOXINS/CT
L5            14 SEA FILE=MEDLINE ABB=ON  L1 AND L2
L6           217 SEA FILE=MEDLINE ABB=ON  SORDELLII
L7             9 SEA FILE=MEDLINE ABB=ON  L5 AND L6
L8           145 SEA FILE=MEDLINE ABB=ON  L1 AND L6
L10            9 SEA FILE=MEDLINE ABB=ON  L8 AND (L2 OR L3)
L11          431 SEA FILE=MEDLINE ABB=ON  L1 AND L4
L12            1 SEA FILE=MEDLINE ABB=ON  L11 AND (TRANSLOCATION DOMAIN)
L13           10 SEA FILE=MEDLINE ABB=ON  L7 OR L10 OR L12
L14         4699 SEA FILE=MEDLINE ABB=ON  GLUCOSYLTRANSFERASES/CT
L15            7 SEA FILE=MEDLINE ABB=ON  L1 AND L6 AND L14
L16            1 SEA FILE=MEDLINE ABB=ON  L15 NOT L13


=> d .med


L16  ANSWER 1 OF 1  MEDLINE
AN   96215121     MEDLINE
DN   96215121
TI   UDP-glucose deficiency in a mutant cell line protects against
     glucosyltransferase toxins from Clostridium difficile and Clostridium
     sordellii.
AU   Chaves-Olarte E; Florin I; Boquet P; Popoff M; von Eichel-Streiber C;
     Thelestam M
CS   Microbiology & Tumorbiology Center (MTC), Box 280, Karolinska Institute,
     S-171 77 Stockholm, Sweden.
SO   JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Mar 22) 271 (12) 6925-32.
     Journal code: HIV. ISSN: 0021-9258.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals; Cancer Journals
EM   199609
AB   We have previously isolated a fibroblast mutant cell with high resistance
     to the two Rho-modifying glucosyltransferase toxins A and B of
Clostridium
     difficile. We demonstrate here a low level of UDP-glucose in the mutant,
     which explains its toxin resistance since: (i) to obtain a detectable
     toxin B-mediated Rho modification in lysates of mutant cells, addition of
     UDP-glucose was required, and it promoted the Rho modification
     dose-dependently; (ii) high pressure liquid chromatography analysis of
     nucleotide extracts of cells indicated that the level of UDP-glucose in
     the mutant (0.8 nmol/10(6) cells) was lower than in the wild type (3.7
     nmol/10(6) cells); and (iii) sensitivity to toxin B was restored upon
     microinjection of UDP-glucose. Using the mutant as indicator cell we also
     found that the related Clostridium sordellii lethal toxin is a
     glucosyltransferase which requires UDP-glucose as a cofactor. Like toxin
B
     it glucosylated 21-23-kDa proteins in cell lysates, but Rho was not a
     substrate for lethal toxin.
```

CT Check Tags: Animal; Support, Non-U.S. Gov't
*Bacterial Toxins: TO, toxicity
Cell Line
***Clostridium: ME, metabolism**
*Clostridium difficile: ME, metabolism
Cricetulus
***Glucosyltransferases: TO, toxicity**
GTP-Binding Proteins: ME, metabolism
Hamsters
Microinjections
Mutation
*Uridine Diphosphate Glucose: DF, deficiency

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=> fil biosis

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FROM JANUARY 1969 TO DATE.

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     FILE 'BIOSIS' ENTERED AT 08:14:53 ON 28 AUG 2000
L1           371 S (C OR CLOSTRID? ) (2W) SORDELLII
L2        149100 S LT OR LETHAL TOXIN#
L3          2244 S IMMUNO TOXIN# OR IMMUNOTOXIN#
L4          2595 S GLUCOSYLTRANSFERAS? OR GLYCOSYL TRANSFERAS?
L5         21155 S RAS
L6            66 S TRANSLOCATION DOMAIN#
L7          4601 S CATALYTIC (2A) (DOMAIN# OR ?PEPTIDE?)
L8            48 S L1 AND L2
L9             0 S L1 AND L3
L10           11 S L1 AND L4
L11           20 S L5 AND L1
L12            2 S L1 AND (L6 OR L7)
L13           10 S L10 AND (L2 OR L3 OR L5)
L14           19 S L11 AND (L2 OR L3 OR L4)
L15           22 S L12 OR L13 OR L14

     FILE 'BIOSIS' ENTERED AT 08:20:32 ON 28 AUG 2000

=> d bib ab 1-22


L15  ANSWER 1 OF 22  BIOSIS  COPYRIGHT 2000 BIOSIS
AN   2000:329353  BIOSIS
DN   PREV200000329353
TI   Involvement of a conserved tryptophan residue in the UDP-glucose binding
     of large clostridial cytotoxin glycosyltransferases.
AU   Busch, Christian; Hofmann, Fred; Gerhard, Ralf; Aktories, Klaus (1)
CS   (1) Institut fuer Pharmakologie und Toxikologie der Albert-Ludwigs-
     Universitaet Freiburg, Hermann-Herder-Strasse 5, Freiburg, D-79104
Germany
SO   Journal of Biological Chemistry, (May 5, 2000) Vol. 275, No. 18, pp.
     13228-13234. print.
     ISSN: 0021-9258.
DT   Article
LA   English
```

SL English
AB Large clostridial cytotoxins catalyze the glucosylation of Rho/**Ras** GTPases using UDP-glucose as a cosubstrate. By site-directed mutagenesis of **Clostridium sordellii lethal toxin** and Clostridium difficile toxin B fragments, we identified tryptophan 102, which is located in a conserved region within the **catalytic domain** of all clostridial cytotoxins, to be crucial for UDP-glucose binding. Exchange of Trp-102 with alanine decreased the **glucosyltransferase** activity by about 1,000-fold and blocked cytotoxic activity after microinjection. Replacement of Trp-102 by tyrosine caused a 100-fold reduction in enzyme activity, indicating a partial compensation of the tryptophan function by tyrosine. Decrease in **glucosyltransferase** and glycohydrolase activity was caused predominantly by an increase in the Km for UDP-glucose of these mutants. The data indicate that the conserved tryptophan residue is implicated in the binding of the cosubstrate UDP-glucose by large clostridial cytotoxins. Data bank searches revealed different groups of proteins sharing the recently identified DXD motif (Busch, C., Hofmann, F., Selzer, J., Munro, J., Jeckel, D., and Aktories, K. (1998) J. Biol. Chem. 273, 19566-19572) and a conserved region defined by a tryptophan residue equivalent to Trp-102 of **C. sordellii lethal toxin**. From our findings, we propose a novel family of glycosyltransferases which includes both prokaryotic and eukaryotic proteins.

L15 ANSWER 2 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 2000:173991 BIOSIS
DN PREV200000173991
TI New method to generate enzymatically deficient Clostridium difficile toxin
B as an antigen for immunization.
AU Genth, Harald; Selzer, Joerg; Busch, Christian; Dumbach, Juergen; Hofmann,
Fred; Aktories, Klaus; Just, Ingo (1)
CS (1) Institut fuer Pharmakologie und Toxikologie, Universitaet Freiburg, Hermann-Herder-Str. 5, D-79104, Freiburg Germany
SO Infection and Immunity., (March, 2000) Vol. 68, No. 3, pp. 1094-1101. ISSN: 0019-9567.
DT Article
LA English
SL English
AB The family of the large clostridial cytotoxins, encompassing Clostridium difficile toxins A and B as well as the lethal and hemorrhagic toxins from **Clostridium sordellii**, monoglucosylate the Rho GTPases by transferring a glucose moiety from the cosubstrate UDP-glucose. Here we present a new detoxification procedure to block the enzyme activity by treatment with the reactive UDP-2',3'-dialdehyde to result in alkylation of toxin A and B. Alkylation is likely to occur in the **catalytic domain**, because the native cosubstrate UDP-glucose completely protected the toxins from inactivation and the alkylated toxin competes with the native toxin at the cell receptor. Alkylated toxins are good antigens resulting in antibodies recognizing only the C-terminally located receptor binding domain, whereas formaldehyde treatment resulted in antibodies recognizing both the receptor binding **domain** and the

catalytic domain, indicating that the **catalytic domain** is concealed under native conditions. Antibodies against the native **catalytic domain** (amino acids 1 through 546) and those holotoxin antibodies recognizing the **catalytic domain** inhibited enzyme activity. However, only antibodies against the receptor binding domain protected intact cells from the cytotoxic activity of toxin B, whereas antibodies against the **catalytic domain** were protective only when inside the cell.

L15 ANSWER 3 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:374252 BIOSIS
DN PREV199900374252
TI Rundown of somatodendritic N-methyl-D-aspartate (NMDA) receptor channels in rat hippocampal neurones: Evidence for a role of the small GTPase RhoA.
AU Noerenberg, Wolfgang; Hofmann, Fred; Illes, Peter; Aktories, Klaus; Meyer, Dieter K. (1)
CS (1) Department of Pharmacology, Albert-Ludwigs-University, Hermann-Herder-Strasse 5, D-79104, Freiburg Germany
SO British Journal of Pharmacology, (July, 1999) Vol. 127, No. 5, pp. 1060-1063.
ISSN: 0007-1188.
DT Article
LA English
SL English
AB 1 Actin filament (F-actin) depolymerization leads to the use-dependent rundown of N-methyl-D-aspartate (NMDA) receptor activity in rat hippocampal neurones. Depolymerization is promoted by Ca2+ which enters the cells via NMDA receptor channels. The **ras** homologue (Rho) GTPases (RhoA, Rac1 and Cdc42) promote actin polymerization and thus control the actin cytoskeleton. We have investigated, by means of the whole-cell patch clamp technique, whether the actin fibres which interact with NMDA receptors are controlled by Rho GTPases. 2 In the presence of intracellular ATP which attenuates rundown, the C3 toxin from Clostridium (C.) botulinum was used to inactivate RhoA. Indeed, it enhanced the use-dependent rundown of NMDA-evoked inward currents to a level similar to that obtained in the absence of ATP. 3 **Lethal toxin** from **Clostridium sordellii** which inactivates Rac1 and Cdc42 lacked this effect. 4 We suggest that the function of somatodendritic NMDA receptor channels in rat hippocampal neurones can be modulated by RhoA via its action on F-actin.

L15 ANSWER 4 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:341395 BIOSIS
DN PREV199900341395
TI G-protein-stimulated phospholipase D activity is inhibited by **lethal toxin** from **Clostridium sordellii** in HL-60 cells.
AU El Hadj, Noomen Ben; Popoff, Michel R.; Marvaud, Jean-Christophe; Payrastre, Bernard; Boquet, Patrice; Geny, Blandine (1)
CS (1) INSERM U332, ICGM, 22 rue Mechain, 75014, Paris France
SO Journal of Biological Chemistry, (May 14, 1999) Vol. 274, No. 20, pp. 14021-14031.
ISSN: 0021-9258.
DT Article

LA English
SL English
AB **Lethal toxin** (**LT**) from **Clostridium sordellii** has been shown in HeLa cells to glucosylate and inactivate **Ras** and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that **LT** treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time-and dose-dependent manner after an overnight treatment with **LT**. A similar dose response to the toxin was found when PLD activity was stimulated by phorbol 12-myristate 13-acetate via the protein kinase C pathway. The toxin effect on actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota toxin from Clostridium perfringens E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease observed in phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in **LT**-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. **LT** was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, Rac, and RalA, inactivated by **LT** and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as **LT** toxin (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, **LT** treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosolprepared from **LT** -treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase Calpha was decreased after **LT** treatment. We conclude that in HL-60 cells, **lethal toxin** from **C. sordellii**, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity.
Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by **LT** in the different modifications observed in HL-60 cells.

L15 ANSWER 5 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:339071 BIOSIS
DN PREV199900339071
TI Effects of cytotoxic necrotizing factor 1 and **lethal**

toxin on actin cytoskeleton and VE-cadherin localization in human endothelial cell monolayers.
AU Vouret-Craviari, Valerie; Grall, Dominique; Flatau, Gilles; Pouyssegur, Jacques; Boquet, Patrice; Van Obberghen-Schilling, Ellen (1)
CS (1) Centre de Biochimie, CNRS UMR6543, Parc Valrose, 06108, Nice Cedex 2 France
SO Infection and Immunity, (June, 1999) Vol. 67, No. 6, pp. 3002-3008. ISSN: 0019-9567.
DT Article
LA English
SL English
AB Integrity of the vascular endothelium is largely dependent on endothelial cell shape and establishment of intercellular junctions. Certain pathogenic bacterial toxins alter the cytoskeletal architecture of intoxicated cells by modulating the GTPase activity of p21 Rho family proteins. In the present study we have analyzed the effect of Rho-directed toxins on the actin cytoskeleton and monolayer integrity of endothelial cells. We report here that Escherichia coli cytotoxic necrotizing factor 1 (CNF1) activates Rho in human umbilical vein endothelial cells (HUVEC). In confluent monolayers, CNF1 treatment induces prominent stress fiber formation without significantly modifying peripheral localization of VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocks thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme (Clostridium botulinum) eliminates actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. **Lethal toxin** (**Clostridium sordellii**), an inhibitor of Rac as well as **Ras** and Rap, potently disrupts the actin microfilament system and monolayer integrity in HUVEC cultures.

L15 ANSWER 6 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:201354 BIOSIS
DN PREV199900201354
TI **Ras** family proteins: New players involved in the diplotene arrest of Xenopus oocytes.
AU Jessus, Catherine; Rime, Helene; Ozon, Rene (1)
CS (1) Laboratoire de Physiologie de la Reproduction, Universite Pierre-et-Marie-Curie, lnra/CNRS ESA 7080, Boite 13, 4, place Jussieu, 75252, Paris cedex 05 France
SO Biology of the Cell (Paris), (Nov., 1998) Vol. 90, No. 8, pp. 573-583. ISSN: 0248-4900.
DT General Review
LA English
AB Oogonia undergo numerous mitotic cell cycles before completing the last DNA replication and entering the meiotic prophase I. After chromosome pairing and chromatid exchanges between paired chromosomes, the oocyte I remains arrested at the diplotene stage of the first meiotic prophase. Oocyte growth then occurs independently of cell division; indeed, during this growth period, oocytes (4n DNA) are prevented from completing the meiotic divisions. How is the prophase arrest regulated? One of the

players of the prophase block is the high level of intracellular cAMP, maintained by an active adenylate cyclase. By using **lethal toxin** from **Clostridium sordellii** (**LT**), a glucosyl-transferase that glucosylates and inactivates small G proteins of the **Ras** subfamily, we have shown that inhibition of either **Ras** or Rap or both proteins is sufficient to release the prophase block of Xenopus oocytes in a cAMP-dependent manner. The implications of **Ras** family proteins as new players involved in the prophase arrest of Xenopus oocytes will be discussed here.

L15 ANSWER 7 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:88114 BIOSIS
DN PREV199900088114
TI Inhibition of small G proteins by **Clostridium sordellii** **lethal toxin** activates cdc2 and MAP kinase in Xenopus oocytes.
AU Rime, Helene; Talbi, Nabila; Popoff, Michel R.; Suziedelis, Kestutis; Jessus, Catherine; Ozon, Rene (1)
CS (1) Laboratoire Physiologie Reproduction INRA/ESA-CNRS 7080, Universite Pierre Marie Curie, Boite 13, 4 Place Jussieu, 75252 Paris Cedex 5 France
SO Developmental Biology, (Dec. 15, 1998) Vol. 204, No. 2, pp. 592-602. ISSN: 0012-1606.
DT Article
LA English
AB The **lethal toxin** (**LT**) from **Clostridium sordellii** is a **glucosyltransferase** that modifies and inhibits small G proteins of the **Ras** family, **Ras** and Rap, as well as Rac proteins. **LT** induces cdc2 kinase activation and germinal vesicle breakdown (GVBD) when microinjected into full-grown Xenopus oocytes. Toxin B from Clostridium difficile, that glucosylates and inactivates Rac proteins, does not induce cdc2 activation, indicating that proteins of the **Ras** family, **Ras** and/or Rap, negatively regulate cdc2 kinase activation in Xenopus oocyte. In oocyte extracts, **LT** catalyzes the incorporation of (14C)glucose into a group of proteins of 23 kDa and into one protein of 27 kDa. The 23-kDa proteins are recognized by anti-Rap1 and anti-Rap2 antibodies, whereas the 27-kDa protein is recognized by several anti-**Ras** antibodies and probably corresponds to K-**Ras**. Microinjection of **LT** into oocytes together with UDP-(14C)glucose results in a glucosylation pattern similar to the in vitro glucosylation, indicating that the 23- and 27-kDa proteins are in vivo substrates of **LT**. In vivo time-course analysis reveals that the 27-kDa protein glucosylation is completed within 2 h, well before cdc2 kinase activation, whereas the 23-kDa proteins are partially glucosylated at GVBD. This observation suggests that the 27-kDa **Ras** protein could be the in vivo target of **LT** allowing cdc2 kinase activation. Interestingly, inactivation of **Ras** proteins does not prevent the phosphorylation of c-Raf1 and the activation of MAP kinase that occurs normally around GVBD.

L15 ANSWER 8 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:18807 BIOSIS
DN PREV199900018807
TI Activation of Ca2+-dependent K+ current in mouse fibroblasts by

lysophosphatidic acid requires a pertussis toxin-sensitive G protein and **Ras**.
AU Repp, Holger; Koschinski, Andreas; Decker, Katrin; Dreyer, Florian (1)
CS (1) Rudolf-Buchheim-Inst. Pharmakol., Justus-Liebig-Univ. Giessen, Frankfurter Strasse 107, D-35392 Giessen Germany
SO Naunyn-Schmiedeberg's Archives of Pharmacology, (Nov., 1998) Vol. 358, No. 5, pp. 509-517.
ISSN: 0028-1298.
DT Article
LA English
AB Lysophosphatidic acid (LPA) is a bioactive lipid that acts through G protein-coupled plasma membrane receptors and mediates a wide range of cellular responses. Here we report that LPA activates a K+ current in NIH3T3 mouse fibroblasts that leads to membrane hyperpolarization. The activation occurs with an EC50 value of 1.7 nM LPA. The K+ current is Ca2+-dependent, voltage-independent, and completely blocked by the K+ channel blockers charybdotoxin, margatoxin, and iberiotoxin with IC50 values of 1.7, 16, and 62 nM, respectively. The underlying K+ channels possess a single channel conductance of 33 pS in symmetrical K+ solution. Pretreatment of cells with pertussis toxin (PTX), **Clostridium sordellii lethal toxin**, or a farnesyl protein transferase inhibitor reduced the K+ current amplitude in response to LPA to about 25% of the control value. Incubation of cells with the protein tyrosine kinase inhibitor genistein or microinjection of the neutralizing anti-**Ras** monoclonal antibody Y13-259 reduced it by more than 50%. In contrast, the phospholipase C inhibitor U-73122 and the protein kinase A activator 8-bromo-cAMP had no effect. These results indicate that the K+ channel activation by LPA is mediated by a signal transduction pathway involving a PTX-sensitive G protein, a protein tyrosine kinase, and **Ras**. LPA is already known to activate Cl- channels in various cell types, thereby leading to membrane depolarization. In conjunction with our results that demonstrate LPA-induced membrane hyperpolarization by activation of K+ channels, LPA appears to be significantly involved in the regulation of the cellular membrane potential.

L15 ANSWER 9 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:390068 BIOSIS
DN PREV199800390068
TI A common motif of eukaryotic glycosyltransferases is essential for the enzyme activity of large clostridial cytotoxins.
AU Busch, Christian; Hofmann, Fred; Selzer, Joerg; Munro, Sean; Jeckel, Dieter; Aktories, Klaus (1)
CS (1) Institut fuer Pharmakologie und Toxikologie der Albert-Ludwigs-Universitaet Freiburg, Hermann-Herder-Str. 5, 79104 Freiburg Germany
SO Journal of Biological Chemistry, (July 31, 1998) Vol. 273, No. 31, pp. 19566-19572.
ISSN: 0021-9258.
DT Article
LA English
AB A fragment of the N-terminal 546 amino acid residues of **Clostridium sordellii lethal toxin** possesses full enzyme activity and glucosylates Rho and **Ras** GTPases in vitro. Here we identified several amino acid residues in **C. sordellii lethal toxin** that are

essential for the, enzyme activity of the active toxin fragment. Exchange of aspartic acid at position 286 or 288 with alanine or asparagine decreased **glucosyltransferase** activity by about 5000-fold and completely blocked glycohydrolase activity. No enzyme activity was detected with the double mutant D286A/D288A. Whereas the wild-type fragment of **C. sordellii lethal toxin** was labeled by azido-UDP-glucose after UV irradiation, mutation of the DXD motif prevented radiolabeling. At high concentrations (10 mM) of manganese ions, the transferase activities of the D286A and D288A mutants but not that of wild-type fragment were increased by about 20-fold. The exchange of Asp270 and Arg273 reduced **glucosyltransferase** activity by about 200-fold and blocked glycohydrolase activity. The data indicate that the DXD motif, which is highly conserved in all large clostridial cytotoxins and also in a large number of glycosyltransferases, is functionally essential for the enzyme activity of the toxins and may participate in coordination of the divalent cation and/or in the binding of UDP-glucose.

L15 ANSWER 10 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:342093 BIOSIS
DN PREV199800342093
TI Functional consequences of monoglucosylation of Ha-**Ras** at effector domain amino acid threonine 35.
AU Herrmann, Christian; Ahmadian, Mohammad Reza; Hofmann, Fred; Just, Ingo (1)
CS (1) Institut fuer Pharmakologie und Toxikologie, Universitaet Freiburg, Hermann-Herder-Strasse 5, D-79104 Freiburg Germany
SO Journal of Biological Chemistry, (June 26, 1998) Vol. 273, No. 26, pp. 16134-16139.
ISSN: 0021-9258.
DT Article
LA English
AB Monoglucosylation of low molecular mass GTPases is an important post-translational modification by which microbes interfere with eukaryotic cell signaling. Ha-**Ras** is monoglucosylated at effector domain amino acid threonine 35 by **Clostridium sordellii lethal toxin**, resulting in a blockade of the downstream mitogen-activated protein kinase cascade. To understand the molecular consequences of this modification, effects of glucosylation on each step of the GTPase cycle of **Ras** were analyzed. Whereas nucleotide binding was not significantly altered, intrinsic GTPase activity was markedly decreased, and GTPase stimulation by the GTPase-activating protein p120GAP and neurofibromin NF-1 was completely blocked, caused by failure to bind to glucosylated **Ras** . Guanine nucleotide exchange factor (Cdc25)catalyzed GTP loading was decreased, but not completely inhibited. A dominant-negative property of modified **Ras** to sequester exchange factor was not detectable. However, the crucial step in downstream signaling, **Ras**-effector coupling, was completely blocked. The Kd for the interaction between **Ras**-GTP and the **Ras**-binding domain of Raf was 15 nM, whereas glucosylation increased the Kd to >1 mM. Because the affinity of **Ras**-GDP for Raf (Kd = 22 muM) is too low to allow functional interaction, a glucose moiety at threonine 35 of **Ras** seems to block completely the interaction with Raf. The net effect of **lethal toxin**-catalyzed glucosylation of **Ras** is the complete blockade of **Ras** downstream signaling.

L15 ANSWER 11 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:270180 BIOSIS
DN PREV199800270180
TI Rho protein inhibition blocks protein kinase C translocation and activation.
AU Hippenstiel, Stefan (1); Kratz, Thomas (1); Kruell, Matthias (1); Seybold, Joachim (1); Eichel-Streiber, Christoph V.; Suttorp, Norbert (1)
CS (1) Dep. Intern. Med., Justus-Liebig-Univ., D-35392 Giessen Germany
SO Biochemical and Biophysical Research Communications, (April 28, 1998) Vol. 245, No. 3, pp. 830-834.
ISSN: 0006-291X.
DT Article
LA English
AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using Clostridium difficile toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and **Clostridium sordellii lethal toxin** -1522 (TcsL) for inactivation of **Ras**-proteins (**Ras** /Rac/Ral,Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as determined by PKC-activity measurements and Western blots for PKCalpha. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L15 ANSWER 12 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:268391 BIOSIS
DN PREV199800268391
TI Activation of a Ca2+-dependent K+ current by the oncogenic receptor protein tyrosine kinase v-Fms in mouse fibroblasts.
AU Decker, Katrin; Koschinski, Andreas; Trouliaris, Sylvia; Tamura, Teruko; Dreyer, Florian; Repp, Holger (1)
CS (1) Rudolf-Buchheim-Institut fuer Pharmakologie, Justus-Liebig-Universitaet Giessen, Frankfurter Strasse 107, D-35392 Giessen Germany
SO Naunyn-Schmiedeberg's Archives of Pharmacology, (April, 1998) Vol. 357, No. 4, pp. 378-384.
ISSN: 0028-1298.
DT Article
LA English
AB We investigated the effects of the receptor-coupled protein tyrosine kinase (RTK) v-Fms on the membrane current properties of NIH3T3 mouse fibroblasts. We found that v-Fms, the oncogenic variant of the macrophage colony-stimulating factor receptor c-Fms, activates a K+ current that is absent in control cells. The activation of the K+ current was Ca2+-dependent, voltage-independent, and was completely blocked by the K+ channel blockers charybdotoxin, margatoxin and iberiotoxin with IC50

values of 3 nM, 18 nM and 76 nM, respectively. To identify signalling components that mediate the activation of this K+ current, NIH3T3 cells that express different mutants of the wild-type v-Fms receptor were examined. Mutation of the binding site for the **Ras** -GTPase-activating protein led to a complete abolishment of the K+ current. A reduction of 76% and 63%, respectively, was observed upon mutation of either of the two binding sites for the growth factor receptor binding protein 2. Mutation of the ATP binding lobe, which disrupts the protein tyrosine kinase activity of v-Fms, led to a 55% reduction of the K+ current. Treatment of wild-type v-Fms cells with **Clostridium sordellii lethal toxin** or a farnesyl protein transferase inhibitor, both known to inhibit the biological function of **Ras**, reduced the K+ current amplitude to 17% and 6% of the control value, respectively. This is the first report showing that an oncogenic RTK can modulate K+ channel activity. Our results indicate that this effect is dependent on the binding of certain **Ras**-regulating proteins to the v-Fms receptor and is not abolished by disruption of its intrinsic protein tyrosine kinase activity. Furthermore, our data suggest that **Ras** plays a key role for K+ channel activation by the oncogenic RTK v-Fms.

L15 ANSWER 13 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:226086 BIOSIS
DN PREV199800226086
TI Specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii lethal toxin** and Clostridium difficile toxin B-1470 in HEK-293 cells.
AU Schmidt, Martina; Voss, Matthias; Thiel, Markus; Bauer, Bettina; Grannass, Andreas; Tapp, Eva; Cool, Robbert H.; Gunzburg, Jean De; Von Eichel-Streiber, Christoph; Jakobs, Karl H. (1)
CS (1) Institut fuer Pharmakologie, Universitaetsklinikum Essen, Hufelandstrasse 55, D-45122 Essen Germany
SO Journal of Biological Chemistry, (March 27, 1998) Vol. 273, No. 13, pp. 7413-7422.
ISSN: 0021-9258.
DT Article
LA English
AB Activation of m3 muscarinic acetylcholine receptor (mAChR), stably expressed in human embryonic kidney (HEK)-293 cells, leads to phospholipase D (PLD) stimulation, a process apparently involving Rho GTPases, as shown by studies with Clostridium botulinum C3 exoenzyme and Clostridium difficile toxin B (TcdB). Direct activation of protein kinase C (PKC) by phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), also induces PLD stimulation, which is additive to the mAChR action and which is only poorly sensitive to inactivation of Rho proteins by TcdB. To study whether **Ras**-like GTPases are involved in PLD regulation, we studied the effects of the TcdB variant TcdB-1470 and **Clostridium sordellii lethal toxin** (TcsL), known to inactivate Rac and. some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by mAChR or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time- and concentration-dependent manner, without alteration in

immunologically detectable PKC isozyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addition of recombinant Racl, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addition of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addition of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TscL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddition of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

L15 ANSWER 14 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:167461 BIOSIS
DN PREV199800167461
TI Chimeric clostridial cytotoxins: Identification of the N-terminal region involved in protein substrate recognition.
AU Hofmann, Fred; Busch, Christian; Aktories, Klaus (1)
CS (1) Inst. Pharmakologie und Toxikologie der Albert-Ludwigs-Univ. Freiburg, Hermann-Herder-Str. 5, 79104 Freiburg Germany
SO Infection and Immunity, (March, 1998) Vol. 66, No. 3, pp. 1076-1081. ISSN: 0019-9567.
DT Article
LA English
AB **Clostridium sordellii lethal toxin** is a member of the family of large clostridial cytotoxins that glucosylate small GTPases. In contrast to Clostridium difficile toxins A and B, which exclusively modify Rho subfamily proteins, **C. sordellii lethal toxin** also glucosylates **Ras** subfamily proteins. By deletion analysis and construction of chimeric fusion proteins of **C. sordellii lethal toxin** and C. difficile toxin B, we localized the enzyme activity of the **lethal toxin** to the N terminus of the holotoxin and identified the region involved in protein substrate specificity. The toxin fragment of the N-terminal 546 amino acid residues of **C. sordellii lethal toxin** glucosylated Rho and **Ras** subfamily proteins, as the holotoxin did. Deletion of a further 30 amino acid residues from the C. terminus of this active fragment drastically reduced glucotransferase activity and blocked glucohydrolase activity. Exchange of amino acid residues 364 through 516 of **lethal toxin** for those in the active toxin B fragment (1 to 546) allowed glucosylation of **Ras** subfamily proteins. In contrast, the chimera with amino acids 1 to 364 from toxin B,

365 to 468 from **lethal toxin**, and 469 to 546 from toxin B exhibited markedly reduced modification of **Ras** subfamily proteins, whereas modification of Rac and Cdc42 was hardly changed. The data indicate that the region of amino acid residues 364 through 516 primarily defines the substrate specificity of **C. sordellii lethal toxin**.

L15 ANSWER 15 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:401209 BIOSIS
DN PREV199799700412
TI Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase.
AU Fiorentini, Carla (1); Fabbri, Alessia; Flatau, Gilles; Donelli, Gianfranco; Matarrese, Paola; Lemichez, Emmanuel; Falzano, Loredana; Boquet, Patrice
CS (1) Dep. Ultrastructures, Ist. Superiore Sanita, Viale Regina Elena 299, 00161 Rome Italy
SO Journal of Biological Chemistry, (1997) Vol. 272, No. 31, pp. 19532-19537.
ISSN: 0021-9258.
DT Article
LA English
AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein toxin from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the toxin into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of Rac, Cdc42, **Ras**, or Rab6) as demonstrated by a discrete increase in the apparent molecular weight of the molecule. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficile toxin B, which inactivates Rho but not those of **Clostridium sordellii LT** toxin, which inhibits **Ras** and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-associated phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PIP-2) nor the phosphatidylinositol 3,4-bisphosphate (PI 3,4-P-2) or 3,4,5-trisphosphate (PIP-3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a toxin that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.

L15 ANSWER 16 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:38161 BIOSIS
DN PREV199799330149
TI Difference in protein substrate specificity between hemorrhagic toxin and **lethal toxin** from **Clostridium sordellii**.
AU Genth, Harald; Hofmann, Fred; Selzer, Joerg; Rex, Gundula; Aktories, Klaus; Just, Ingo (1)

CS (1) Inst. Pharmakol. Toxikol., Albert-Ludwigs-Univ. Freiburg, Hermann-Herder-Str. 5, D-79104 Freiburg Germany
SO Biochemical and Biophysical Research Communications, (1996) Vol. 229, No. 2, pp. 370-374.
ISSN: 0006-291X.
DT Article
LA English
AB The hemorrhagic toxin (HT) from **Clostridium sordellii** is pharmacologically related to Clostridium difficile toxins A and B and **Clostridium sordellii lethal toxin** which have been recently identified as mono-glucosyl-transferases. Here we report that HT, which is coexpressed with **lethal toxin**, is also a **glucosyltransferase**. Whereas **lethal toxin** glucosylates the Rho subfamily proteins Rac and Cdc42 and the **Ras** subfamily proteins H-**Ras** and Rap, the substrate specificity of HT is strictly confined to the Rho subfamily proteins Rho, Rac and Cdc42. Comparable to **lethal toxin**, transferase activity of HT is stimulated by Mn-2+. Acceptor amino acid in Rho was identified by mutagenesis as threonine-37. **C. sordellii** HT is a novel member of the family of clostridial mono-glucosyl-transferases, a family which modified the Rho and **Ras** GTPases.

L15 ANSWER 17 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:529529 BIOSIS
DN PREV199699251885
TI The **Ras**-related protein Ral is monoglucosylated by **Clostridium sordellii lethal toxin**.
AU Hofmann, Fred; Rex, Gundula; Aktories, Klaus; Just, Ingo (1)
CS (1) Inst. Pharmakol. Toxikol., Albert-Ludwigs-Univ. Freiburg, Hermann-Herder-Str. 5, D-79104 Freiburg Germany
SO Biochemical and Biophysical Research Communications, (1996) Vol. 227, No. 1, pp. 77-81.
ISSN: 0006-291X.
DT Article
LA English
AB **Clostridium sordellii lethal toxin** (**LT**), a cytotoxin which causes preferential destruction of the actin cytoskeleton, has been recently identified as **glucosyltransferase** to modify the low molecular mass GTPases Rac, **Ras** and Rap. We report here on **LT** produced by **C. sordellii** strain 6018 which glucosylates in addition to Rac, **Ras** and Rap the Rai protein. **LT** from strain VPI9048 however does not glucosylate Rai. Besides recombinant Rai, cellular Ral is also substrate. In the GDP-bound form, Ral is a superior substrate to the GTP form. Acceptor amino acid for glucose is threonine-46 which is equivalent to threonine-35 in H-**Ras** located in the effector region. The Ral-glucosylating toxin is a novel isoform of **Ras**-modifying clostridial cytotoxins.

L15 ANSWER 18 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:304295 BIOSIS
DN PREV199699026651
TI **Clostridium sordellii lethal toxin** is a Mn-2+-dependent **glucosyltransferase**.

AU Genth, H. (1); Selzer, J. (1); Green, G. A.; Aktories, K. (1); Just, I. (1)
CS (1) Inst. Pharmakologie Toxikologie, univ. Freiburg, D-79104 Freiburg Germany
SO Naunyn-Schmiedeberg's Archives of Pharmacology, (1996) Vol. 353, No. 4 SUPPL., pp. R20.
Meeting Info.: 37th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology Mainz, Germany March 12-14, 1996
ISSN: 0028-1298.
DT Conference
LA English

L15 ANSWER 19 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:304294 BIOSIS
DN PREV199699026650
TI Inactivation of **Ras** by glucosylation catalyzed by **Clostridium sordellii lethal toxin**.
AU Just, I. (1); Selzer, J. (1); Kern, O. (1); Green, G. A.; Aktories, K. (1)
CS (1) Inst. Pharmakologie Toxikologie, Univ. Freiburg, D-79104 Freiburg Germany
SO Naunyn-Schmiedeberg's Archives of Pharmacology, (1996) Vol. 353, No. 4 SUPPL., pp. R19.
Meeting Info.: 37th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology Mainz, Germany March 12-14, 1996
ISSN: 0028-1298.
DT Conference
LA English

L15 ANSWER 20 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:267362 BIOSIS
DN PREV199698823491
TI **Ras**, Rap, and Rac small GTP-binding proteins are targets for **Clostridium sordellii lethal toxin** glucosylation.
AU Popoff, Michel R.; Chaves-Olarte, Esteban; Lemichez, Emmanuel; Von Eichel-Streiber, Christoph; Thelestam, Monica; Chardin, Pierre; Cussac, Didier; Antonny, Bruno; Chavrier, Philippe; Flatau, Gilles; Giry, Murielle; De Gunzburg, Jean; Boquet, Patrice (1)
CS (1) Inst. Pasteur, Unite des Toxines Microbiennes, 75724 Paris, Cedex 15 France
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 17, pp. 10217-10224.
ISSN: 0021-9258.
DT Article
LA English
AB **Lethal toxin** (**LT**) from **Clostridium sordellii** is one of the high molecular mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that **LT** is a **glucosyltransferase** that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. **LT** glucosylates **Ras**, Rap, and Rac. In **Ras**, threonine at position 35 was identified as the target amino acid glucosylated by **LT**. Other related members of the **Ras** GTPase superfamily,

including RhoA, Cdc42, and Rab6, were not modified by **LT**. Incubation of serum-starved Swiss 3T3 cells with **LT** prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the toxin blocks **Ras** function in vivo. We also demonstrate that **LT** acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphology. **LT** is thus a powerful tool to inhibit **Ras** function in vivo.

L15 ANSWER 21 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:267361 BIOSIS
DN PREV199698823490
TI Inactivation of **Ras** by **Clostridium sordellii lethal toxin**-catalyzed glucosylation.
AU Just, Ingo (1); Selzer, Joerg; Hofmann, Fred; Green, Gaynor A.; Aktories, Klaus
CS (1) Inst. Pharmakologie und Toxikologie der Univ. Freiburg, Hermann-Herder-Strasse 5, D-79104 Freiburg Germany
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 17, pp. 10149-10153.
ISSN: 0021-9258.
DT Article
LA English
AB The **lethal toxin** (**LT**) from **Clostridium sordellii** belongs to the family of large clostridial cytotoxins causing morphological alterations in cultured cell lines accompanied by destruction of the actin cytoskeleton. **C. sordellii LT** exhibits 90% homology to Clostridium difficile toxin B, which has been recently identified as a monoglucosyltransferase (Just, I., Selzer, J., Wilm, M., von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) Nature 375, 500-503). We report here that **LT** too is a **glucosyltransferase**, which uses UDP-glucose as cosubstrate to modify low molecular mass GTPases. **LT** selectively modifies Rac and **Ras**, whereas the substrate specificity of toxin B is confined to the Rho subfamily proteins Rho, Rac, and Cdc42, which participate in the regulation of the actin cytoskeleton. In Rac, both toxin B and **LT** share the same acceptor amino acid, threonine 35. Glucosylation of **Ras** by **LT** results in inhibition of the epidermal growth factor-stimulated p42/p44 MAPkinase signal pathway. **LT** is the first bacterial toxin to inactivate **Ras** in intact cells.

L15 ANSWER 22 OF 22 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:193493 BIOSIS
DN PREV199698749622
TI UDP-Glucose deficiency in a mutant cell line protects against **glucosyltransferase** toxins from Clostridium difficile and **Clostridium sordellii**.
AU Chaves-Olarte, Esteban; Florin, Inger; Boquet, Patrice; Popoff, Michel; Von Eichel-Streiber, Christoph; Thelestam, Monica (1)
CS (1) Microbiol. and Tumorbiology Cent., Box 280, Karolinska Inst., S-17177 Stockholm Sweden
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 12, pp. 6925-6932.
ISSN: 0021-9258.

DT Article
LA English
AB We have previously isolated a fibroblast mutant cell with high resistance to the two Rho-modifying **glucosyltransferase** toxins A and B of Clostridium difficile. We demonstrate here a low level of UDP-glucose in the mutant, which explains its toxin resistance since: (i) to obtain a detectable toxin B-mediated Rho modification in lysates of mutant cells, addition of UDP-glucose was required, and it promoted the Rho modification dose-dependently; (ii) high pressure liquid chromatography analysis of nucleotide extracts of cells indicated that the level of UDP-glucose in the mutant (0.8 nmol/10-6 cells) was lower than in the wild type (3.7 nmol/106 Cells); and (iii) sensitivity to toxin B was restored upon microinjection of UDP-glucose. Using the mutant as indicator cell we also found that the related **Clostridium sordellii lethal toxin** is a **glucosyltransferase** which requires UDP-glucose as a cofactor. Like toxin B it glucosylated 21-23kDa proteins in cell lysates, but Rho was not a substrate for **lethal toxin**.